REMARKS

Claims 3-4, 17, 57-63 and 65-66 are pending. As previously requested, rejoinder of withdrawn claims 5-8, 10, 15, 35-47 and 64 would be appreciated upon an indication that an elected product claim is allowable.

1. Isolated conjugates of claims 3-4, 17, 57-63 and 65-66 have substantial, specific, and credible utility.

With respect to the utility rejection, Applicants respectfully traverse. As explained in the response to the previous Office Action, a person skilled in the art would readily recognize the utility of proteins, their fragments, and derivatives that were ubiquitylated via the N-end rule pathway (together, "compounds") as providing standards, markers, and controls for assays and other processes related to ubiquitylation and proteasome-mediated degradation pathways. As discussed in the Background section of the specification, defects in such pathways are well-known in the prior art to cause different human diseases. This is not speculation but proven facts.

First, the skilled artisan would readily recognize from the teachings in Applicants' specification that the claimed compounds provide the products of ubiquitylation and substrates for proteasome-mediated degradation. They are related to metabolism in normal, healthy humans as well as pathogenesis of disease.

Second, the skilled artisan would readily recognize the utility of such compounds as standards and controls for quantifying accumulation of ubiquitylated products in organisms, cells, or cell-free assays. Conversely, the compounds may also be used as standards and controls for quantifying consumption of ubiquitylated products in organisms, cells, or cell-free assays by proteasome-mediated degradation.

Third, the skilled artisan would readily recognize the utility of such compounds as markers for comparison to or identification of naturally-occurring products of ubiquitylation in organisms, cells, or cell-free assays. Conversely, the compounds may also be used as markers for comparison to or identification of naturally-occurring substrates in organisms, cells, or cell-free assays of proteasome-mediated degradation.

Fourth, prior art that would be known to the skilled artisan teach these pathways are targets for drug therapy (see, for example, the enclosed abstract of Wong et al., 8 *Drug Discovery Today* 746, Aug 2003). Applicants' specification also teaches at page 3, "The defective regulation of the ubiquitin-proteasome system manifests in diseases that range from developmental abnormalities and autoimmunity to neurodegenerative diseases and cancer." Defects in either enzymes belonging to these pathways or their substrates have been identified as causing human diseases (see the Background section, especially page 6 et seq., of the specification and the enclosed Glickman & Ciechanover, 82 *Physiological Reviews* 373, 2002, especially pages 409-16).

The skilled artisan would also recognize that it might be unnecessary to use the full-length ubiquitylated protein to study a biochemical pathway. Instead, a fragment or derivative could be used for convenience. For example, a fragment that preserves a linear epitope will be recognized by an antibody to that particular epitope and can serve as a standard or control. Because of their usefulness in assaying these compounds, the claimed compounds would also be useful for analyzing the effectiveness of drugs or other therapies against N-end rule accumulation of ubiquitylated products and their proteasomal degradation.

Therefore, a skilled artisan would find credible Applicants' asserted utilities of providing products of the ubiquitylation pathway and substrates of the proteasomal degradation pathway as specific and substantial utilities for diagnosing human disease or screening drug candidates for treatment of human disease which do not unduly interfere with essential physiological pathways.

It was alleged on page 4 of the Action that "the specification does not describe a specific and substantial utility for the conjugate of ubiquitin or ubiquitin derivative with the respective proteins or the composition comprising the conjugate." Drug candidates to treat disease may be screened by their effect on the proteins, fragments, or derivatives. Ubiquitylated products would accumulate by the action of the ubiquitylation pathway and they would degrade by the action of the proteasome. Drugs that inhibit or stimulate these pathways would cause a corresponding increase or decrease in the

amount of the compounds. Use of the compounds as standards, markers, and controls for assays and other processes related to ubiquitylation and proteasome-mediated degradation pathways such as those causing the human diseases described above is a specific and credible utility of screening drug candidates. This utility is not dependent as the Examiner contends on page 4 of the Action on (i) whether the compounds are "naturally present" fragments or derivatives or (ii) whether the compounds inhibit the ubiquitylation process. As noted above, fragments or derivatives of a natural substrate may be used as starting material for proteasome-mediated degradation or they may be further processed versions of the natural substrate. They could be more convenient to use than the natural substrate because fragments could possibly be easier to handle due to their size (e.g., more soluble or less labile under storage conditions) and derivatives could be conjugated to tags for purification or labels for visualization. Finally, "the protein already conjugated with ubiquitin" is not required to inhibit the ubiquitylation process (although it might through feedback inhibition) because it is starting material for the next pathway of proteasomal degradation.

As clearly stated in the *Manual of Patent Examining Procedures* (M.P.E.P.):

> Practical considerations require the Office to rely on the inventor's understanding of his or her invention in determining whether and in what regard an invention is believed to be "useful." Because of this, Office personnel should focus on and be receptive to assertions made by the applicant that an invention is "useful" for a particular reason.

M.P.E.P. § 2107.01 at 2100-22.

> In most cases, an applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement of Section 101.

M.P.E.P. § 2107.02 at 2100-30. As the Court of Custom and Patent Appeals held:

> [A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.

In re Marzocchi, 169 USPQ 367, 369 (C.C.P.A. 1971) (emphasis in original). And the Federal Circuit has used this standard for determining compliance with Section 101. See *In re Brana*, 34 USPQ2d 1436,1441 (Fed. Cir. 1995).

Thus, the M.P.E.P. and case law direct the Office to presume that a statement of utility made by an applicant is true. Favorable reconsideration of the Section 101 is requested. If this rejection is maintained, and to narrow the grounds on which an appeal would be argued, the Examiner is respectfully requested to clarify which of the three criteria is alleged to be deficient: whether or not Applicants' asserted utility is (i) specific to the claimed invention, (ii) substantial for benefiting the public, and/or (iii) credible.

2. Claims 3-4, 17, 57-63 and 65-66 are fully enabled by the disclosure in the specification.

With respect to the enablement rejection, Applicants respectfully traverse. It is believed that a satisfactory rebuttal of the Examiner's contentions in the utility rejection would result in withdrawal of both pending rejections. But to the extent that a separate rebuttal of his *Wands* analysis is possible, those arguments are addressed below. Applicants' specification provides extensive disclosure of the claimed compounds and protocols to prepare and characterize the claimed compounds. Assays to ubiquitylate and/or to degrade proteins are also known in the prior art. The skilled artisan would be able to produce the claimed compounds and to make/use them as described by Applicants' in their specification and above using general knowledge known in the art.

Detailed procedures for making and using an invention may not be necessary if the description of the invention itself is sufficient to permit those skilled in the art to make and use the invention. M.P.E.P. § 2164. A patent does not teach, and preferably omits, what is well known in the art. *In re Buchner*, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech v. Monoclonal Antibodies*, 231 USPQ 81, 94 (Fed. Cir. 1986); *Lindemann Maschinenfabrik v. American Hoist & Derrick*, 221 USPQ 481, 489 (Fed. Cir. 1984).

The Examiner based his rejection on the alleged unpredictability in the art with regard to the tertiary structure required for the interaction of substrate with the cognate E3 ubiquitin-protein ligase enzymes. But a person skilled in the art would be able to practice the subject invention without undue experimentation in view of the disclosure in the specification and further in view of the high level of skill in the relevant art. As shown by working examples in Applicants' specification, the substrates of the claims were successfully ubiquitylated (and in some cases processed by a protease to expose an N-degron) in the context of in vitro assays. Thus, undue experimentation would not be required to use these substrates in a reaction involving ubiquitylation and/or proteolysis.

The "enablement" requirement of Section 112 is satisfied by objective standards. Whether this is achieved by illustrative examples or by broad terminology is not important. *In re Marzocchi*, 169 USPQ 367, 369 (C.C.P.A. 1971). Enablement is not negated by having to exercise some degree of experimentation such as routine optimization of protocols, but the experimentation needed to practice the invention must not be undue. The key word is undue, not experimentation. *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (emphasis added).

The experimentation required to practice the full scope of the claimed invention is reasonable and not undue because a person skilled in the art regularly engages in such experimentation. The specification enables a skilled artisan to create protein fragments of the disclosed sequence and to conduct N-end rule pathway ubiquitylation screening to determine whether a fragment is ubiquitylated via the N-end rule pathway. The specification also enables a skilled artisan to synthesize proteins, to derivatize them, and to conduct N-end rule pathway ubiquitylation screening to determine whether a derivative is ubiquitylated via the N-end rule pathway. The in vitro N-end rule ubiquitylation screen disclosed in Applicants' specification at page 40, lines 11-13, allows a skilled artisan to rapidly evaluate any fragments or derivatives: "The activity of these proteins as ubiquitylation substrates can be deter-mined by measuring the accumulation of ubiquitylated products" (see also the working examples on pages 76-89 of the specification).

Applicants' specification also provides detailed guidance at page 43, lines 9-22, to a skilled artisan by defining the claimed fragments and derivatives, and by providing numerous examples at pages 41-42 of ubiquitylated fragments of the claimed proteins characterized by gel electrophoresis (see also Examples 2-6 for molecular weight and cleavage position estimates for protein fragments, and their corresponding images in Figures 2-7 for gel electrophoretic analysis). Derivatives may be made by protocols known in the prior art for attaching purification tags and/or visualization labels.

Such experimentation is not undue. A person skilled in the art would, therefore, have a significant amount of confidence and information available to guide them in selecting and predicting the activity of ubiquitin fragments and derivatives.

> The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.

In re Wands, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

> The test for enablement is whether one reasonably skilled in the art to make or use the invention from the disclosure in the patent coupled with information known in the art without undue experimentation. A patent may be enabling even though some experimentation is necessary.

United States v. Telectronics, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988).

Favorable reconsideration of the Section 112 rejection is requested.

Applicants earnestly solicit an early Notice of Allowance. If any further information is needed, the Examiner is invited to contact the undersigned.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

Gary R. Tanigawa
Reg. No. 43,180

901 North Glebe Road, 11th Floor
Arlington, VA 22203-1808
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

1: Drug Discov Today. 2003 Aug 15;8(16):746-54.

Drug discovery in the ubiquitin regulatory pathway.

Wong BR, **Parlati F**, **Qu K**, **Demo S**, **Pray T**, **Huang J**, **Payan DG**, **Bennett MK**.

Rigel Pharmaceuticals, 1180 Veterans Blvd South San Francisco, CA 94080, USA. bwong@rigel.com

The ubiquitin system has been implicated in the pathogenesis of numerous disease states, including oncogenesis, inflammation, viral infection, CNS disorders and metabolic dysfunction. Ubiquitin conjugation and deconjugation to substrate proteins is carried out by multiple families of proteins, each with a defined role in the enzymatic cascade. This conjugation-deconjugation system parallels the kinase-phosphatase system in that both alter protein function by the addition and removal of post-translational modifiers. Our understanding of ubiquitin biology and strategies to interfere pharmacologically with the ubiquitin regulatory machinery is progressing rapidly. In light of increased interest in ubiquitin pathways as drug targets, we review the ubiquitin enzymatic cascades, highlighting therapeutic opportunities and enzymatic mechanisms. We also discuss the challenges of targeting this class of enzymes with small molecules, as well as current approaches and progress in drug discovery.

PMID: 12944097 [PubMed - indexed for MEDLINE]

Physiol Rev
82: 373–428, 2002; 10.1152/physrev.00027.2001.

The Ubiquitin-Proteasome Proteolytic Pathway: Destruction for the Sake of Construction

MICHAEL H. GLICKMAN AND AARON CIECHANOVER

Faculty of Biology and the Institute for Catalysis Science and Technology, and Department of Biochemistry, Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Glickman, Michael H., and Aaron Ciechanover. The Ubiquitin-Proteasome Proteolytic Pathway: Destruction for the Sake of Construction. *Physiol Rev* 82: 373–428, 2002; 10.1152/physrev.00027.2001.—Between the 1960s and 1980s, most life scientists focused their attention on studies of nucleic acids and the translation of the coded information. Protein degradation was a neglected area, considered to be a nonspecific, dead-end process. Although it was known that proteins do turn over, the large extent and high specificity of the process, whereby distinct proteins have half-lives that range from a few minutes to several days, was not appreciated. The discovery of the lysosome by Christian de Duve did not significantly change this view, because it became clear that this organelle is involved mostly in the degradation of extracellular proteins, and their proteases cannot be substrate specific. The discovery of the complex cascade of the ubiquitin pathway revolutionized the field. It is clear now that degradation of cellular proteins is a highly complex, temporally controlled, and tightly regulated process that plays major roles

in a variety of basic pathways during cell life and death as well as in health and disease. With the multitude of substrates targeted and the myriad processes involved, it is not surprising that aberrations in the pathway are implicated in the pathogenesis of many diseases, certain malignancies, and neurodegeneration among them. Degradation of a protein via the ubiquitin/proteasome pathway involves two successive steps: *1*) conjugation of multiple ubiquitin moieties to the substrate and *2*) degradation of the tagged protein by the downstream 26S proteasome complex. Despite intensive research, the unknown still exceeds what we currently know on intracellular protein degradation, and major key questions have remained unsolved. Among these are the modes of specific and timed recognition for the degradation of the many substrates and the mechanisms that underlie aberrations in the system that lead to pathogenesis of diseases.

I. INTRODUCTION AND OVERVIEW OF UBIQUITIN-MEDIATED PROTEOLYSIS

Like all macromolecular components of an organism, the proteome is in a dynamic state of synthesis and degradation. During proteolysis, the peptide bonds that link amino acids are hydrolyzed, and free amino acids are released. The process is carried out by a diverse group of enzymes termed proteases. During proteolysis, the energy invested in the synthesis of the peptide bond is released. Distinct proteolytic mechanisms serve different physiological requirements and allow the organism to accommodate to changing environmental and pathophysiological conditions.

One should distinguish between destruction of "foreign" and "self" proteins. Foreign dietary proteins are degraded "outside" the body, in the lumen of the gastrointestinal tract. To avoid triggering an immune response, the epithelial lining of the digestive tract does not allow absorption of intact proteins into the body, and they are degraded to nonantigenic amino acids that are absorbed by the body and serve as building blocks for synthesis of its own proteins. Self proteins can also be classified into extracellular and intracellular; the two groups of proteins are degraded via two distinct mechanisms. Extracellular proteins such as the blood coagulation factors, immunoglobulins, albumin, cargo-carrying proteins [such as the core protein of the low-density lipoprotein (LDL)], and peptide hormones (such as insulin) are taken up via pinocytosis or receptor-mediated endocytosis. They are then carried via a series of vesicles (endosomes) that fuse with primary lysosomes where they are degraded. During this process, the extracellular proteins are never exposed to the intracellular environment (the cytosol) and remain "extracellular" (topologically) throughout. Degradation of proteins in lysosomes is not specific, and all engulfed proteins exposed to lysosomal proteases are degraded at approximately the same rate.

Several observations lead to the prediction that degradation of intracellular proteins must be carried out by completely distinct mechanisms. The process is highly specific, and different proteins have half-life times that vary from a few minutes (e.g., the tumor suppressor p53) to several days (e.g., the muscle proteins actin and myosin) and up to a few years (crystalline). Furthermore, inhibitors of lysosomal degradation, weak bases such as chloroquine, for example, do not have any effect on degradation of intracellular proteins under basal metabolic conditions. These compounds titrate the normal acidic intralysosomal pH and bring it to a point that does not allow activity of the lysosomal proteases. These findings led to the hypothesis that degradation of intracellular proteins must be carried out by a nonlysosomal proteolytic system that is endowed with a high degree of specificity toward its substrates. Also, the fact that the proteolytic enzymes and their substrates reside in the same cellular compartment predicted a requirement for tightly regulated machinery that uses metabolic energy for control. The discovery of the ubiquitin-proteasome proteolytic pathway has resolved these enigmas.

Degradation of a protein via the ubiquitin-proteasome pathway involves two discrete and successive steps: *1*) tagging of the substrate by covalent attachment of multiple ubiquitin molecules and *2*) degradation of the tagged protein by the 26S proteasome complex with release of free and reusable ubiquitin. This last process is mediated by ubiquitin recycling enzymes [deubiquitinating enzymes (DUBs); see sect. VIII]. Conjugation of ubiquitin, a highly evolutionarily conserved 76-residue polypeptide, to the protein substrate proceeds via a three-step cascade mechanism (Fig. 1). Initially, the ubiquitin-activating enzyme E1 activates ubiquitin in an ATP-requiring reaction to generate a high-energy thiol ester intermediate, E1-S~ubiquitin. One of several E2 enzymes [ubiquitin-carrier proteins or ubiquitin-conjugating enzymes (UBCs)] transfers the activated ubiquitin moiety from E1, via an additional high-energy thiol ester intermediate, E2-S~ubiquitin, to the substrate that is specifically bound to a member of the ubiquitin-protein ligase family, E3. There are a number of different classes of E3 enzymes (see sect. II and Figs. 1 and 2). For the HECT (homologous to the E6-AP COOH terminus) domain E3s, the ubiquitin is transferred once again from the E2 enzyme to an active site Cys residue on the E3, to generate a third high-energy thiol ester intermediate, ubiquitin~S-E3, before its transfer to the ligase-bound substrate. RING finger-containing E3s catalyze direct transfer of the activated ubiquitin moiety to the E3-bound substrate. E3s catalyze the last step in the conjugation process: covalent

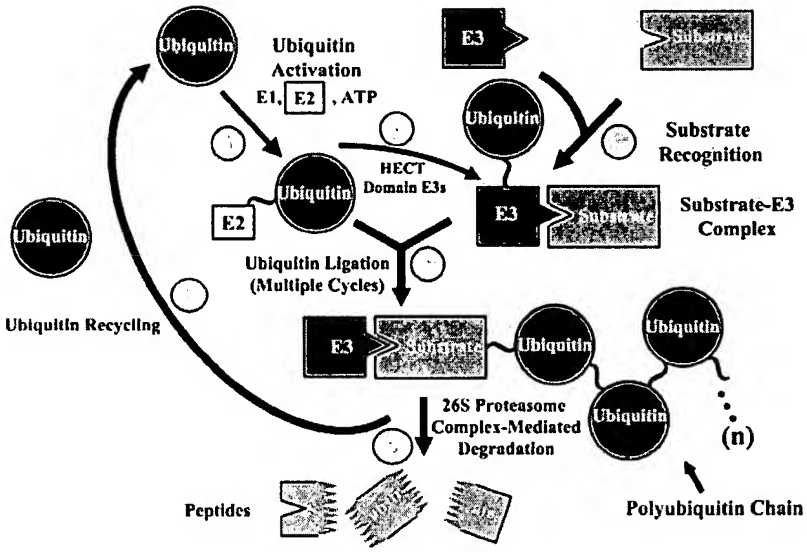

FIG. 1. The ubiquitin proteolytic pathway. *1*: Activation of ubiquitin by the ubiquitin-activating enzyme E1, a ubiquitin-carrier protein, E2 (ubiquitin-conjugating enzyme, UBC), and ATP. The product of this reaction is a high-energy E2~ubiquitin thiol ester intermediate. *2*: Binding of the protein substrate, via a defined recognition motif, to a specific ubiquitin-protein ligase, E3. *3*: Multiple (*n*) cycles of conjugation of ubiquitin to the target substrate and synthesis of a polyubiquitin chain. E2 transfers the first activated ubiquitin moiety directly to the E3-bound substrate, and in following cycles, to previously conjugated ubiquitin moiety. Direct transfer of activated ubiquitin from E2 to the E3-bound substrate occurs in substrates targeted by RING finger E3s. *3'*: As in *3*, but the activated ubiquitin moiety is transferred from E2 to a high-energy thiol intermediate on E3, before its conjugation to the E3-bound substrate or to the previously conjugated ubiquitin moiety. This reaction is catalyzed by HECT domain E3s. *4*: Degradation of the ubiquitin-tagged substrate by the 26S proteasome complex with release of short peptides. *5*: Ubiquitin is recycled via the activity of deubiquitinating enzymes (DUBs).

attachment of ubiquitin to the substrate. The ubiquitin molecule is generally transferred to an ϵ-NH_2 group of an internal Lys residue in the substrate to generate a covalent isopeptide bond. In some cases, however, ubiquitin is conjugated to the NH_2-terminal amino group of the substrate. By successively adding activated ubiquitin moieties to internal Lys residues on the previously conjugated ubiquitin molecule, a polyubiquitin chain is synthesized (Fig. 1). The chain is recognized by the downstream 26S proteasome complex (see sect. VI). Thus E3s play a key role in the ubiquitin-mediated proteolytic cascade since they serve as the specific recognition factors of the system.

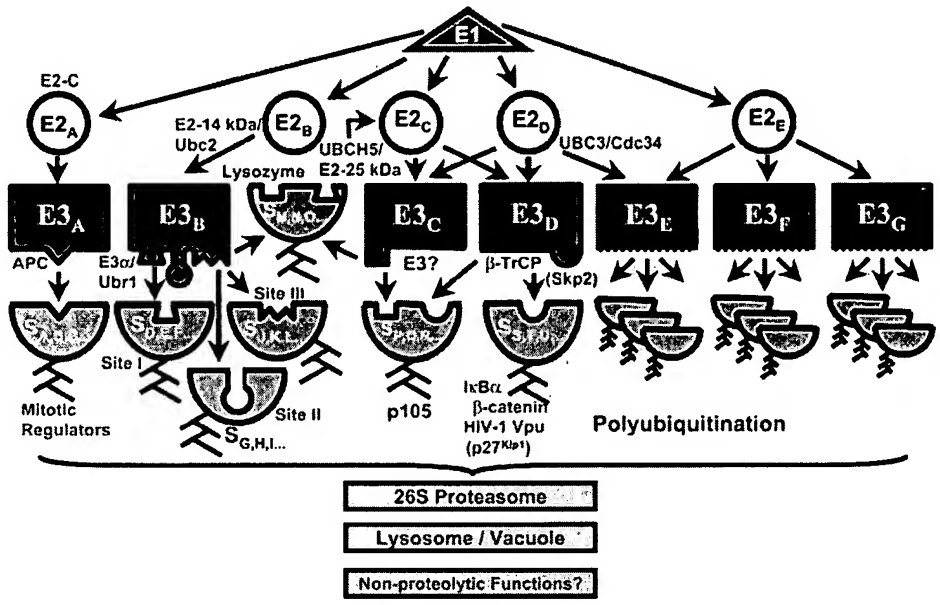

FIG. 2. The hierarchical structure of the ubiquitin system. The simplified view of the hierarchical structure of the ubiquitin conjugation machinery is that a single E1 (red) activates ubiquitin for all conjugation reactions. E1 interacts with all E2s (yellow). Typically, each E2, exemplified by $E2_E$, interacts with several E3s ($E3_E$, $E3_F$, and $E3_G$; blue). Each E3 targets several substrates (green). The interactions of the conjugating enzymes among themselves and with many of the target substrates may differ from this "classical" cascade. For example, a single E3 can interact with 2 distinct E2s ($E3_E$, for example, interacts with $E2_D$ and $E2_E$). Also, a single E3 ($E3_B$, for example) can have several distinct recognition sites targeting different classes of substrates ($S_{D,E,F}$, $S_{G,H,I}$, $S_{J,K,L}$, and $S_{M,N,O}$). Finally, some substrates can be targeted by different E3s, recognizing different motifs ($S_{M,N,O}$ and $S_{P,Q,R}$). It should be noted that not all recognition cascades have been demonstrated experimentally, and some are still putative. Also, because of the complexity of the scheme and space constraints, only a few examples could be brought, and we could not assign distinct enzymes to defined substrates. Thus the ligase ($E3_C$) that ubiquitinates lysozyme ($S_{M,N,O...}$) is not necessarily the same ligase that ubiquitinates, in the cell, p105 ($S_{P,Q,R...}$), as one may conclude from the scheme (see sect. III for details).

The proteasome is a large, 26S, multicatalytic protease that degrades polyubiquitinated proteins to small peptides. It is composed of two subcomplexes: a 20S core particle (CP) that carries the catalytic activity and a regulatory 19S regulatory particle (RP). The 20S CP is a barrel-shaped structure composed of four stacked rings, two identical outer α-rings and two identical inner β-rings (Fig. 5). The eukaryotic α- and β-rings are composed each of seven distinct subunits, giving the 20S complex the general structure of $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$. The catalytic sites are localized to some of the β-subunits. Each extremity of the 20S barrel can be capped by a 19S RP. One important function of the 19S RP is to recognize ubiquitinated proteins and other potential substrates of the proteasome. A ubiquitin-binding subunit of the 19S RP has indeed been identified; however, its importance and mode of action have not been discerned. A second function of the 19S RP is to open an orifice in the α-ring that will allow entry of the substrate into the proteolytic chamber. Also, because a folded protein would not be able to fit through the narrow proteasomal channel, it is assumed that the 19S particle unfolds substrates and inserts them into the 20S CP. Both the channel opening function and the unfolding of the substrate require metabolic energy, and indeed, the 19S RP contains six different ATPase subunits. After degradation of the substrate, short peptides derived from the substrate are released, as well as reusable ubiquitin (for general schemes, see Figs. 1 and 5).

A major unresolved question is, How does the system achieve its high specificity and selectivity? Why are certain proteins extremely stable in the cell, whereas others are extremely short-lived? Why are certain proteins degraded only at a particular time point during the cell cycle or only after specific extracellular stimuli, yet they are stable under most other conditions? It appears that specificity of the ubiquitin system is determined by two distinct and unrelated groups of proteins: *1*) E3s and *2*) ancillary proteins. First, within the ubiquitin system, substrates must be specifically recognized by an appropriate E3 as a prerequisite to their ubiquitination. In most cases however, substrates are not recognized in a constitutive manner and are not recognized directly by the E3. In some instances, the E3 must "be switched on" by undergoing posttranslational modification to yield an active form that recognizes the substrate (Fig. 3). In many other cases, it is the substrate that must undergo a certain change that renders it susceptible for recognition (Fig. 3). The stability of additional proteins depends on association with ancillary proteins such as molecular chaperones that act as recognition elements in *trans* and serve as a link to the appropriate ligase. Others, such as certain transcription factors, have to dissociate from the specific DNA sequence to which they bind in order to be recognized by the system. Stability of yet other proteins depends on oligomerization. Thus, in addition to the E3s themselves, modifying enzymes (such as kinases), ancillary proteins, or DNA sequences to which substrates bind, also play an important role in the recognition process.

Ubiquitin-mediated proteolysis of a variety of cellular proteins plays an important role in many basic cellular processes. Among these are regulation of cell cycle and division, differentiation and development, involvement in the cellular response to stress and extracellular effectors, morphogenesis of neuronal networks, modulation of cell surface receptors, ion channels and the secretory pathway, DNA repair, transcriptional regulation, transcriptional silencing, long-term memory, circadian rhythms, regulation of the immune and inflammatory responses, and biogenesis of organelles. The list of cellular proteins that are targeted by ubiquitin is growing rapidly. Among them are cell cycle regulators such as cyclins, cyclin-dependent kinase inhibitors, and proteins involved in sister chromatid separation, tumor suppressors, as well as transcriptional activators and their inhibitors. Cell surface receptors and endoplasmic reticulum (ER) proteins are also targeted by the system. Finally, mutated and denatured/misfolded proteins are recognized specifically and are removed efficiently. In this capacity, the system is a key player in the cellular quality control and defense mechanisms.

With the numerous substrate proteins targeted and the multitude of processes involved, it is not surprising that aberrations in the ubiquitin system have been implicated in the pathogenesis of many inherited and acquired human pathologies. In some cases, the linkage between the proteolytic system and the resulting pathology is direct, whereas in others it is less obvious. It is impossible to cover in a single review, or even in a comprehensive monograph, all that we currently know of the ubiquitin system, its enzymatic components, ancillary proteins, modes of action, mechanisms of substrate recognition, modes of regulation, and most of all, the numerous processes it is involved in or the consequences of aberrations in its activity. We therefore decided to bring to the reader an updated view of the general components of the system, the conjugating enzymes and the proteasome, explain their mode of action, and highlight, via specific examples, several of the processes in which the system is involved. We will end by describing in greater detail the recently evolving field of the involvement of this system in pathogenesis of human diseases. For more details about the ubiquitin system, the reader is referred to the many specific reviews written on it recently. On ubiquitin ligases, see References 82, 198, 207, 333, 350, 449, 468; on proteasomes, see References 37, 124, 458; and for recent monographs on the ubiquitin system, see References 170 and 343.

II. THE UBIQUITIN CONJUGATING MACHINERY: E1, E2, AND E3

A. The Ubiquitin-Activating Enzyme, E1

E1 activates ubiquitin, via a two-step intramolecular and ATP-dependent reaction, to generate a high-energy E1-thiol-ester~ubiquitin intermediate (Fig. 1). The activated ubiquitin moiety is then transferred to E2 (163). The yeast genome encodes for a single ubiquitin-activating enzyme, *UBA1*. Inactivation of this gene is lethal (302). The protein contains a nuclear localization signal (149, 302). The enzyme is phosphorylated, a modification that was suggested to play a role in its cell cycle-dependent nuclear localization (422). However, the physiological relevance of this modification has not been further substantiated.

B. Ubiquitin-Carrier Proteins (Ubiquitin-Conjugating Enzymes), E2s

E2s catalyze covalent attachment of ubiquitin to target proteins, or, when acting along with HECT domain E3s, transfer of the activated ubiquitin moiety to a high-energy E3~ubiquitin intermediate. They all share an active-site ubiquitin-binding Cys residue and are distinguished by the presence of a UBC domain required for binding of distinct E3s. In a few cases, they can also interact with the substrate (217). The physiological significance of this interaction is not known. Eleven ubiquitin conjugating enzymes (Ubc1–8, 10, 11, 13) have been identified in the yeast genome. Two additional enzymes, Ubc9 and Ubc12, are members of the UBC family, although they conjugate the ubiquitin-like proteins Smt3 and Rub1, respectively, and not ubiquitin (see sect. IX). Many more E2s have been described in higher organisms. Typically, each E2 interacts with a number of ligases, thus being involved in targeting numerous substrates (for hierarchy of the ubiquitin pathway, see Fig. 2 and below).

Yeast Ubc2/Rad6 acts along with Ubr1/E3α to target N-end rule substrates (478), but this pair can also ubiquitinate many other substrates targeted by other motifs (132, 366). Another E2, Ubc3/Cdc34, acts along with the different permutations of the SCF complex (see below), targeting mostly phosphorylated substrates (82). Ubc4 and Ubc5 are involved in targeting the bulk of short-lived/abnormal/misfolded proteins (403). Ubc1 functionally overlaps with Ubc4 and Ubc5 but appears to act primarily in the early stages of growth after germination of spores (404). These three enzymes constitute a family essential for cell growth and viability.

Ubc6 is an ER membrane-anchored E2, while Ubc7 is a membrane-associated E2 (34, 416) recruited to the ER membrane by Cue1 (35). They are involved in degradation of proteins from within the ER (23, 353), but surprisingly, also of soluble proteins such as the transcription factor MATα2 (58). Ubc8 (357) is involved in catabolite inactivation of fructose-1,6-bisphosphatase (394); however, inactivation of the gene does not lead to any detectable phenotype. Ubc10 is involved in peroxisome biogenesis (470), but the underlying mechanism(s) is still obscure. Ubc11 acts along with the cyclosome/anaphase promoting complex (APC), which is the E3 that targets cell cycle regulators (333). Ubc13, similar to Ubc2/Rad6, is a member of the error-free postreplication repair pathway in *Saccharomyces cerevisiae* (48). It forms a specific heteromeric complex with Mms2p, a complex that is required for assembly of polyubiquitin chains linked through Lys^{63}. Mms2p is a ubiquitin-conjugating enzyme variant (UEV) protein that resembles E2s but lacks the defining E2 active site Cys residue. A deletion mutant Δ*ubc13* yeast strain is ultraviolet (UV) sensitive, and it is possible that the noncanonical, novel polyubiquitin chains signal in DNA repair (179).

The number and variety of different E2s in mammalian species is much greater. For example, BRUCE (BIR repeat-containing ubiquitin-conjugating enzyme) is a colossal (528 kDa) E2 isolated from mice (154). It is membrane associated and localizes to the Golgi apparatus. Remarkably, in addition to being an active E2, BRUCE bears a baculovirus inhibitor of apoptosis repeat (BIR) motif, which has been identified exclusively in apoptosis inhibitors of the IAP-related protein family. The BIR motifs of IAP proteins are indispensable for their anti-cell death activity and function probably through protein-protein interaction. This suggests that BRUCE may combine properties of IAP-like proteins and E2, and it is possible that the two activities are related. Interestingly, certain ubiquitin ligases, E3s, are IAPSs and have been shown to modulate apoptosis (483a). They are RING-finger ligases (see below) that have an autoubiquitinating activity. They are degraded after apoptotic stimuli and target pro-apoptotic proteins for degradation in the nonapoptosing cell.

The terminology of the different E2 enzymes is confusing, and similar terms given to yeast and mammalian enzymes do not reflect functional or structural homology. Thus human UBCH1 (215) is not the human homolog of yeast Ubc1, but rather the homolog of yeast Ubc2/Rad6. Similarly, yeast Ubc11 is the homolog of clam E2-C, *Xenopus* UBCx, and human E2-H10, all four partners with the APC E3 complex (440, 441). Yeast ER Ubc6 and Ubc7 are not the homologs of human UbcH6 and UbcH7 that are soluble enzymes involved in targeting of soluble proteins in the cytosol (321).

C. Ubiquitin-Protein Ligases, E3s

The E3s, which are responsible for the specific recognition of the multitude substrates of the ubiquitin system,

are the least defined components of the pathway and display the greatest variety among its different components. The ubiquitin ligase is a protein or a protein complex that binds to both the E2 and the substrate. Interaction with the substrate can be direct or via ancillary proteins. In most cases (i.e., RING finger domain E3s, see below), the E3 serves as a scaffold that brings together the E2 and the substrate to the proximity that allows for efficient transfer of the activated ubiquitin moiety from E2 to the substrate. In other cases (HECT domain E3s), the activated ubiquitin is transferred from E2 to an internal Cys residue on E3 before conjugation of ubiquitin to an NH_2 group in the target. Here, the E3 has a catalytic role. An additional subset of E3s (U-box domain) termed also E4s serves as scaffold to aid in transfer of ubiquitin from the E2 to a previously conjugated ubiquitin moiety, in effect elongating polyubiquitin chains (see however below).

Sites of ubiquitination vary among different substrates. For most proteins, the first ubiquitin moiety is conjugated to an ϵ-NH_2 group of an internal Lys residue. For at least three substrates, the transcription factor MyoD (45), the latent membrane protein 1 (LMP1) of the Epstein-Barr virus (EBV) (18), and the E7 oncoprotein of the human papillomavirus (HPV) (365), it has been shown that the first ubiquitin moiety is attached to the free α-NH_2 terminus of the protein. In either class, additional ubiquitin moieties are then conjugated to an ϵ-NH_2 group of an internal Lys residue in the previously conjugated ubiquitin. As for the location of the Lys residues that are tagged in the target protein, no rules can be formulated. For signal-induced degradation of IκBα, it has been shown that the polyubiquitin chain is conjugated specifically to either Lys-21 or Lys-22 (391). The same residues can also be sumoylated, possibly protecting the inhibitor from ubiquitination and subsequent degradation (83). For p53, multiple Lys residues that reside in a limited region in the COOH-terminal domain (K372, K373, K381, and K382) are targeted by the E3 enzyme Mdm2 (317); substitution of all of them decreased ubiquitination significantly. Interestingly, the same Lys residues are also targeted by acetyl groups, and it appears that acetylation and ubiquitination play opposite roles in governing the stability of the tumor suppressor. In contrast, for cyclin B (227) and the ζ-chain of the T cell receptor (TCR) (184), mutagenic analyses indicate that there is no specificity as for the Lys residue targeted, and no single residue serves as a specific anchor for the polyubiquitin chain.

Another hurdle that E3s must clear is their association with upstream and downstream elements of the system. Such links are important to ensure efficiency and processivity of the proteolytic process. It has been shown that RING finger E3s bind, via their RING finger domain, to their partner E2s (198, 207, 449, 500). Another important interaction could be between the ligases and the proteasome. One can assume that the binding site on the proteasome for the polyubiquitin chains attached to target substrates may be sufficient, and proteins, once conjugated, detach from the conjugation machinery and attach to the proteasome. However, a more efficient machinery could be one in which the conjugation machinery itself associates with the proteasome. It has been recently shown that two human homologs of the yeast ubiquitin-like protein Dsk2, hPLIC-1 and hPLIC-2, associate with both proteasomes and ubiquitin ligase complexes. Overexpression of hPLIC proteins interferes with cellular degradation of two unrelated ubiquitin-proteasome substrates, p53 and IκBα, but not of a ubiquitin-independent substrate, ODC (236). The mechanism of inhibition has not been deciphered, and it is not known whether additional proteins are also involved in linking the conjugation and proteolysis machineries. A similar mechanism has been described recently in *S. cerevisiae*, in which an adaptor protein, Cic1, links the proteasome with components of an SCF E3 complex (199). A linkage between ligases and E2s on one hand and proteasomes on the other hand may provide an explanation for the rapid and efficient signal- and cell cycle-induced degradation of key regulatory cellular proteins that possibly occurs in a processive manner.

Because of lack of significant homology among the ligases initially identified, it was thought that they belong to a large number of protein families. Recently, it has become clear that even though E3s are heterogeneous, they can nevertheless be classified into two major groups: HECT domain- and RING finger-containing E3s and several minor groups.

1. HECT domain E3s

HECT domain proteins harbor a 350-amino acid residue sequence homologous to the COOH-terminal domain of the prototypical member of the family E6-AP (E6-associated protein) (190, 191). This domain contains a conserved Cys residue to which the activated ubiquitin moiety is transferred from E2 (389). The NH_2-terminal domain, which varies among the different HECT domain proteins, is probably involved in specific substrate recognition. The first enzyme described in this family, E6-AP, targets p53 for rapid degradation in the presence of the HPV oncoprotein E6 (388). In the absence of viral ancillary protein, E6-AP targets for degradation other native cellular proteins, such as Blk, a member of the Src family of kinases (146, 323). Mutations in E6-AP have been implicated in the pathogenesis of Angelman syndrome, a severe form of inherited mental and motor retardation (229). Under these conditions, the yet to be identified target substrate that accumulates is probably toxic to the developing brain cells.

NEDD4 is a HECT domain E3 that targets the kidney epithelial Na^+ channel (1, 376), while both basal and induced degradation of the yeast uracil permease, FUR4,

is mediated by the Npi1/Rsp5 HECT domain ligase (117). The transforming growth factor (TGF)-β family of proteins regulates many different biological processes, including cell growth and differentiation. TGF-β ligands signal across cell membrane through type I and type II serine/threonine-kinase receptors, which in turn activate the SMAD signaling pathway. Inside the cell, a receptor-regulated group of SMADs is phosphorylated by the receptor kinases. In this way, receptors for different factors are able to pass on specific signals along the pathway. Thus receptors for bone morphogenetic protein (BMP) signal via SMADs 1, 5, and 8, whereas receptors for activin and TGF-β signal via SMADs 2 and 3. The SMAD proteins serve therefore as key signaling effectors for the TGF-β superfamily of growth factors. Phosphorylation of SMADs leads to their association with Smad4, the "common partner" SMAD, which results in translocation of the complex into the nucleus and initiation of specific transcriptional activity. The activity of SMADs must be tightly regulated to ensure timely activity of the different proteins activated under different conditions by distinct ligands. Smurf1, a new member of the HECT family of ligases, selectively interacts with SMADs specific for the BMP pathway (502). Ectopic expression of Smurf1 inhibits the transmission of BMP signals and affects pattern formation in *Xenopus*. Smurf2, also a member of the HECT domain family of E3s, appears to act similarly on SMAD1, though, in high concentration, it decreases also the level of SMAD2 (497). These findings suggest that ubiquitination by Smurf2 may regulate the competence of a cell to respond to TGF-β/BMP signaling through a distinct degradation pathway that is similar to, yet independent of, Smurf1.

2. *RING finger motif-containing E3s*

For many years, RING finger domains were thought to play a role in dimerization of proteins. It is only recently that RING finger domain-containing proteins were identified as ubiquitin ligases, transferring ubiquitin to both heterologous substrates as well as to the RING proteins themselves (198, 207, 280, 449). RING fingers have been defined by a pattern of conserved Cys and His residues that form a cross-brace structure that probably binds two Zn cations $CX_2CX_{(9-39)}CX_{(1-3)}HX_{(2-3)}C/HX_2CX_{(4-48)}CX_2C$ (39, 385). RING finger domains are classified into RING-HC and RING-H2, depending on whether a Cys or His occupies the fifth coordination site, respectively. At least for the HC domain, structural analysis has revealed that it has two interleaved Zinc-binding sites. RING fingers probably function to recruit the E2 component of the ubiquitination machinery. The crystal structure of c-Cbl, a RING finger ligase involved in targeting activated receptor tyrosine kinases (269), bound to a cognate E2 and a kinase peptide (representing the substrate) shows how the RING domain recruits the E2 UbcH7 (500). The E2 binds to the RING through contacts between a groove in the RING and two loops in the E2 fold of UbcH7. The structure reveals a rigid coupling between the peptide-binding and the E2-binding domains and a conserved surface channel leading from the peptide to the E2 active site, suggesting that RING E3s may function as scaffolds that position the substrate and the E2 optimally for ubiquitin transfer (500). Comparison with the HECT domain group reveals that a similar region in the E2 is recognized by a similar spatially organized cleft in the HECT E3, although the latter is clearly distinct in its primary structure from that of the RING finger domain (187).

The RING finger domain-containing E3 family is composed of two distinct groups, single and multisubunit proteins. Certain members, Mdm2 (42, 120, 280), Ubr1/E3α (250, 367), and Parkin (411), for example, are monomers or homodimers and contain both the RING finger domain and the substrate-binding/recognition site in the same molecule. Many others are part of multisubunit complexes. Among them are the APC involved in degradation of cell cycle regulators (333), the von-Hippel Lindau-Elongins B and C (VBC)-Cul2-RING finger complex (196, 276, 338) involved in the degradation of HIF1-α (195, 197, 220, 299), and the Skp1-Cullin/Cdc53-F-box protein (SCF)-RING finger complexes involved in degradation of signal- and cell cycle-induced phosphorylated proteins (82). In some of these complexes, such as in SCF or VBC, the RING finger domain component, Rbx1/Hrt1/Roc1 (413, 430), is involved in E2 recruitment and assembly of other components of the complex, but not in substrate recognition. A different subunit, the F-box protein in SCF, and most probably, the pVHL subunit in VBC, carry out the substrate recognition function. In APC, the RING finger domain protein is Apc11, which has been demonstrated to bind, at least in vitro, also the cyclin Clb2, and to catalyze its ubiquitination (266). However, this experiment was carried out with recombinant Apc11, in the absence of the other complex subunits, and therefore it is not clear that this subunit is the only substrate-binding protein in the complex (see however below).

A) SPECIFIC RING FINGER COMPLEXES: SCF COMPLEXES. The SCF complexes act along with the E2 Cdc34/Ubc3 (82), and possibly with members of the UBCH5 family (131). The catalytic complex may have the following structure: {E2·Hrt1/Rbx1/Roc1·Cdc53/Cullin·Skp1·F-box protein·protein substrate}, such that the E2 is recruited by the RING finger-containing protein and the substrate is bound to the F-box protein. A fifth component in the SCF ligase complex, Sgt1, has been recently described (233); however, its role is not yet clear. The Hrt1/Rbx1/Roc1, Skp1, and members of the Cdc53/Cullin-1 family components are probably common to all SCF complexes. The specific substrate binding F-box protein is the most variable component; therefore, the different complexes are designated

according to the variable F-box component [e.g., SCF^{Cdc4}, $SCF^{\beta\text{-}TrCP}$ (transducin repeat-containing protein), SCF^{Skp2}]. All SCF complexes are probably involved in targeting phosphorylated substrates. $SCF^{\beta\text{-}TrCP}$ targets phosphorylated IκBα (487), β-catenin (234), and HIV-1-Vpu (293). The signal recognized by $SCF^{\beta\text{-}TrCP}$ in all three substrates is DS(P)GψXS(P) (222), although p105 (157, 327), p100, and other, yet to be identified substrates, may utilize slightly different signals (see below). Despite the fact that Vpu undergoes phosphorylation that recruits the E3, it is the CD4 receptor that is ubiquitinated and degraded *in trans* after formation of a CD4·Vpu·SCF ternary complex.

SCF^{Skp2} targets E2F-1 (294) and $p27^{Kip1}$ (52). Although $p27^{Kip1}$ must be phosphorylated on Thr-187 to be recognized, it has not been shown whether recognition of E2F-1 also requires phosphorylation (see below), although it is highly likely that this is the case here too. SCF^{Grr4} in budding yeast (413) targets the phosphorylated form of the G1 cyclin Cln1.

Regulation of the activity of SCF complexes is mediated, in large, via timely phosphorylation of its different substrates. However, an additional layer of regulation may be operational via modulation of the level of the F-box protein component of the complex, its key substrate recognition element. It has been reported (501) that in *S. cerevisiae*, Cdc4, the F-box protein that is involved in targeting cell cycle regulators, is unstable, in contrast to other, commonly shared, components of the complex. Grr1, another F-box component of SCF complexes, is also unstable, and like Cdc4, targeted for degradation by the ubiquitin system. Interestingly, ubiquitination of Cdc4 is mediated by SCF^{Cdc4}. A dominant negative species of Cdc4 that lacks the F-box domain, and therefore cannot recruit the Skp1 component, is stable, suggesting that ubiquitination is catalyzed by Cdc4 in an intramolecular mechanism within the SCF complex. Furthermore, the mutant protein inhibited cell proliferation by interfering, most probably, in the targeting of a variety of cell cycle regulators. This interference may be due not only to the inability of the SCF^{Cdc4} to catalyze ubiquitination of its own substrates because the F-box protein is mutated, but also because of the inability of other F-box proteins to assemble into active SCF complexes, as the other subunits are engaged with the stable Cdc4. Thus the finding that the F-box components are unstable suggests a mechanism of regulating SCF function through ubiquitination and proteolysis of these components. In a different study, Spiegelman et al. (419) presented evidence that β-catenin/TCF signaling elevates the expression of β-TrCP mRNA and protein. Induction of β-TrCP expression by the β-catenin/TCF pathway results in accelerated degradation of the wild-type β-catenin, suggesting that the negative feedback loop regulation may control the β-catenin/TCF pathway.

An interesting case involves the yeast SCF^{Met30} that targets the transcription factor Met4. Surprisingly, Met4 is a relatively stable protein, and its abundance is not influenced by Met30. However, transcriptional repression of Met4 target genes correlates with SCF^{Met30}-dependent ubiquitination of Met4. Functionally, ubiquitinated Met4 associates with target promoters but fails to activate transcription (214). Thus it appears that ubiquitination of transcription factors that does not involve their proteolysis can be utilized to directly regulate their activities. The mechanism of ubiquitination has not been deciphered. It may be similar to Ubc13/Uev1A and TRAF6 (a RING finger protein)-mediated activation of IκB kinase (IKK) that involves generation of a ubiquitin chain assembled via Lys-63, that is not involved in targeting for degradation (79). It has been shown that TRAF6, which may be a ubiquitin ligase, is ubiquitinated following cell signaling, and that ubiquitination of TRAF6 and/or of an additional, yet unidentified, protein(s) is essential for activating the TAK1·TAB1·TAB2 kinase complex. Activation of this complex is required for activation of the downstream IKK. It is not clear whether any component of the TAK1 kinase must be also ubiquitinated during this process (461).

B) VBC. The VBC-Cul2-Rbx1 complex has a structure similar to that of SCF. It contains Elongins B and C, Cullin 2, and pVHL, in addition to Hrt1/Rbx1/Roc1 (219), the same RING finger protein that is also shared by SCF complexes. The substrate binding/recognizing subunit has not been identified, but it is most probably pVHL. One known substrate targeted by this E3 is HIF1-α (195, 197, 220, 299). Similar to the modularity of F-box proteins in SCF complexes, it is possible that more than one substrate recognition protein is involved in VBC·Cul2·Rbx1 E3 complexes. Thus Socs1 was reported to target the hematopoietic specific guanine nucleotide exchange factor VAV for degradation in context of Elongins B and C complex (81).

C) APC. In budding yeast, the APC/cyclosome (333) contains 11 subunits, of which, Apc11 was shown to be the RING finger domain protein, that may also carry the substrate-binding and ubiquitination functions, at least toward certain substrates (266). Apc2 carries a Cullin homology domain and therefore may be related to the Cdc53/Cullin1 component of the SCF complexes (493). Specificity of the ligase complex toward its many mitotic and possibly G_1/S transition substrates is probably determined by a set of substoichiometric regulators that associate with it, such as Cdc20 (Fizzy/CDC20/p55) and Hct1/Cdh1 (Fizzy-related/HCT1). In addition, regulation also involves phosphorylation of APC subunits [such as by Cdc5, the Polo-like kinase (Plk/Plx)] and dephosphorylation (see below). It should be noted that the activity of the APC/cyclosome is not regulated by modulation of the level of its different subunits, as they all appear to be constitutively expressed throughout the cell cycle. As for APC/cyclosome-associated E2 enzymes, APC acts along with Ubc4 (UBC4) and Ubc11 (E2-H10 in human, UBCx in *Xenopus*, and E2-C in clam; see above); however, it is not

known whether the different E2s are specific for distinct substrates, or whether they are interchangeable. Deletion analysis in yeast suggests that Ubc11 and Ubc4/Ubc5 can substitute for one another (441). Ubiquitination of cyclins, but also of other APC substrates, such as Geminin, a *Xenopus* protein involved in the inhibition of DNA replication (301), is mediated by a short, 9-amino acid residue sequence, the "destruction box" (see below). APC also recognizes and ubiquitinates the inhibitors of sister chromatid separation, Pds1 (securin) in *S. cerevisiae*, and Cut 2 in *S. pombe*, via a "destruction box." These proteins are inhibitors of Esp1 (separin), the protease that upon activation (after removal of securin) is involved in cleavage of cohesin, the protein that attaches the two sister chromatids to one another. Ase1, a yeast protein required for elongation of mitotic spindle during mitosis, is also targeted by the APC/cyclosome.

3. E4 and U box-containing proteins

A recently discovered protein is a ubiquitin chain elongation factor, E4. Koegl et al. (241) have shown that efficient multiubiquitination required for proteasomal targeting of a model ubiquitin-fusion substrate utilizes an additional conjugation factor, named E4. This protein is identical to Ufd2 (ubiquitin fusion degradation pathway), involved in targeting of chimeric model substrates with a stable ubiquitin moiety fused to their NH_2 terminus (209). In yeast, E4 binds to the ubiquitin moieties of preformed short conjugates and catalyzes ubiquitin chain elongation in conjunction with E1, E2, and E3. It thus renders them preferred substrates for proteasomal degradation. E4 defines a novel protein family that shares a modified version of the RING finger, designated as U box, that lacks the hallmark metal-chelating residues of the RING finger motif (12). Most of the signature Cys residues of the RING finger are not conserved in the U box, and the structure is probably stabilized by hydrogen bonds and salt bridges. A typical box contains the following residues: xxxx**h**x**s**x**l**xx-**ph**h**x**-**sh**xxxx**s**xxx**hppx**x**I**x**px**h**xxxxxxxx**sP**x**s**xxxxxxxx-xl**x**s**x**xxx**px**xxx, where x denotes any residue, h is a hydrophobic residue, l is an aliphatic residue, and s is small, p is polar, and - is a negatively charged residue. P and I indicate Pro and Ile that appear in more than 90% of identified members of the family.

A number of U-box proteins have been shown to elongate chains dependent on E1 and E2, but independent on E3 (153). Therefore, it is possible that U-box enzymes constitute a subfamily of E3 enzymes that has the ability, together with E1 and E2, to ubiquitinate ubiquitin-protein fusions or elongate short polyubiquitin chains by mediating transfer of ubiquitin to a previously conjugated ubiquitin molecule rather than to the substrate itself, in effect elongating chains (241). An E3 would still be necessary in this scenario to attach the first ubiquitin to the substrate. In addition, they can function also as ubiquitin ligases that act independently of the action of an E3, i.e., target their substrates directly (153). It is not clear yet whether the U box, like the E3 RING finger motif, is involved in recruiting the E2 component of the conjugation machinery, or whether it binds to the short ubiquitin chain conjugated to the target protein. An interesting member of the family is CHIP, which has recently been shown to be involved in the degradation of the cystic fibrosis transmembrane conductance regulator (CFTR) and the glucocorticoid receptor (66, 303). CHIP was shown to interact, through a set of tetratricorepeat motifs, with Hsc70 and Hsp90. In the case of CFTR, it functions with Hsc70 to sense the folded state of CFTR and to target aberrant forms for proteasomal degradation by promoting their ubiquitination. It has been shown the CHIP acts as an independent ubiquitin ligase that targets both Hsc70 (204a) and also other unfolded proteins for degradation (315a). It is possible that CHIP does not have a substrate binding site, but ubiquitinates unfolded proteins that are bound to Hsp70. Thus it is the heterodimer CHIP-Hsp70 that acts as a ubiquitin ligase (75b). The U box appears to be essential for this process because overexpression of CHIP-Δ-U box inhibited the action of endogenous CHIP and blocked CFTR ubiquitination and degradation. In the case of the steroid receptor, CHIP acts along with Hsc90. It is possible that CHIP is the E4 component in the conjugation machinery of CFTR and the steroid receptor, but it can also be the direct E3 ubiquitin ligase.

III. HIERARCHICAL STRUCTURE OF THE UBIQUITIN SYSTEM

The ubiquitin system has a rhomboid-shaped structure, with its tips at the E1 and the proteasome and the broadest point at the recognition plane between the substrates and the E3s (Fig. 2). Thus a single E1 enzyme activates ubiquitin for all conjugation reactions and transfers it to all known E2s (161, 244, 350, 474), and a single enzyme, the proteasome, proteolyses all substrates targeted for degradation by ubiquitination (124, 458). Most E2s interact with several E3s (Fig. 2), and usually, most E3 are found to interact with several different protein substrates via similar or identical recognition motifs. However, this hierarchy is more complicated and cannot be seen simply as a pyramid structure, but rather as a complex network of overlapping interactions between multiple components (Fig. 3). For instance, specific E3s can often interact with more than one E2, and some substrates can be targeted by more than one E3. A few examples highlight the complex combinatorics of different E2-E3-substrate interactions (Fig. 2).

1) A single E2, $E2_A$, acts along with a single E3 ($E3_A$) to target a set of substrates ($S_{A,B,C...}$) that share a common recognition motif. This is probably the case with E2-C/Ubc11/UBCx/UBCH-10 that acts along with the cyclosome/APC in targeting cell cycle regulators that share the destruction box recognition motif (14, 333, 441).

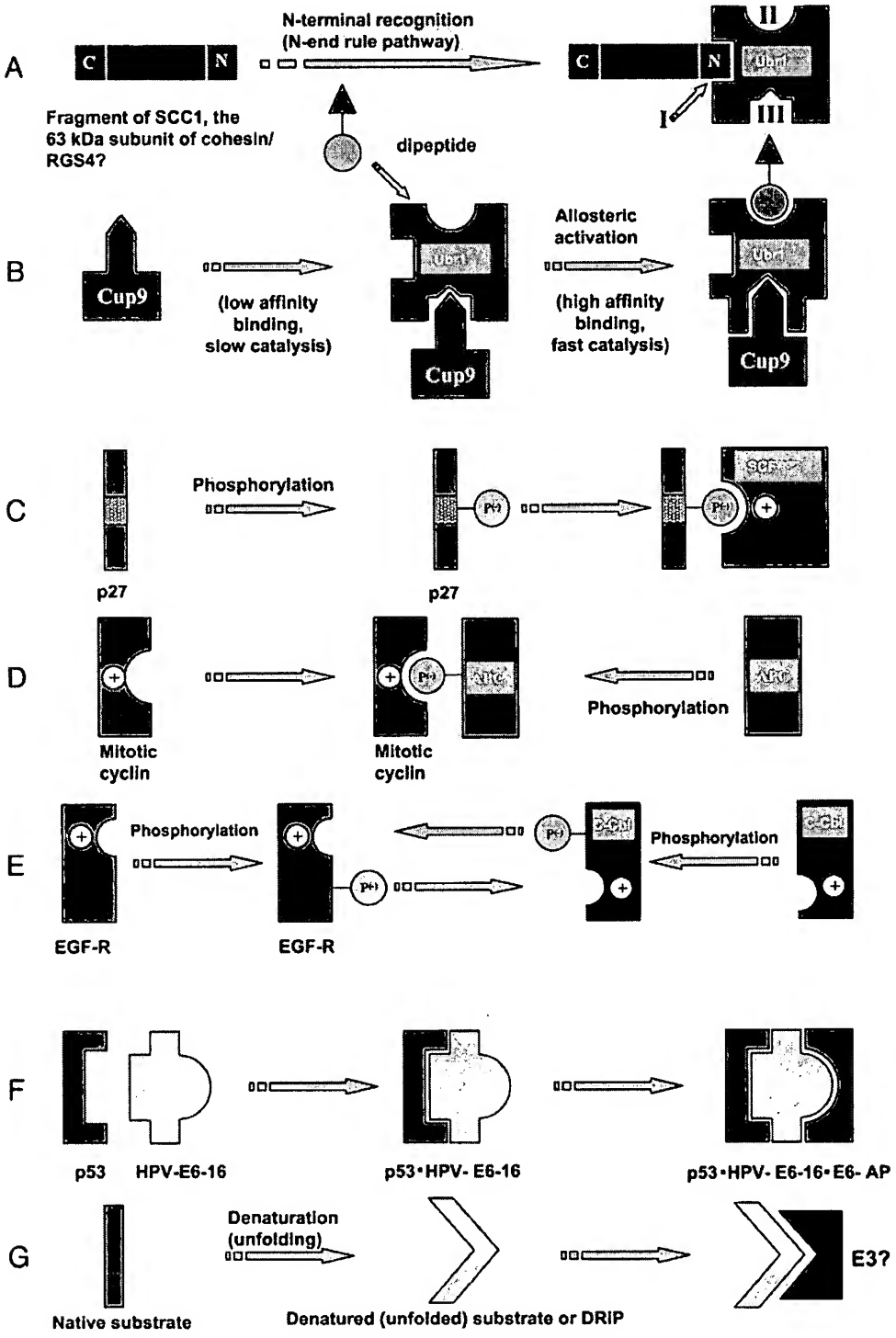

FIG. 3. Modes of recognition of proteolytic substrates by ubiquitin-protein ligases, E3s. Ligases are in red, and substrates are in blue. *A*: recognition of substrates via their NH_2-terminal residue (N-end rule pathway). *B*: peptide-induced allosteric activation of Ubr1. *C*: recognition of phosphorylated substrate. *D*: phosphorylation of the E3 is required for its activity. *E*: phosphorylation of both the ligase and its substrate is required for ubiquitination. *F*: recognition in *trans* via an ancillary protein. *G*: the ubiquitin system degrades selectively abnormal/mutated/un- and misfolded proteins, including also defective ribosomal products, DRiPs (nascent chains that are degraded cotranslationally). EGF-R, epidermal growth factor receptor. For details, see section IV. Recognition via hydroxylated proline is not shown.

2) $E2_B$ interacts with $E3_B$, a ligase that has three distinct recognition sites. Each of these sites recognizes a distinct targeting motif on a defined set of substrates. Thus this E3 can interact independently with three sets of substrates, $S_{D,E,F...}$, $S_{G,H,I...}$, and $S_{J,K,L...}$. This is the case with mammalian E2–14 kDa (351) and its yeast homolog Ubc2 (203), which interact with mammalian E3α (366, 478) and yeast Ubr1 (287), respectively. E3α/Ubr1 has two N-end rule pathway recognition sites; the first recognizes substrates with basic NH_2 termini (site I), whereas the second recognizes substrates with hydrophobic NH_2-terminal residues (site II) (250, 368). However, this ligase also has a third site, a "body" site (site III), that recognizes an as yet to be identified motif(s) residing downstream to the NH_2 terminus (133, 161, 250, 368, 454).

3) Certain substrates contain two different recognition motifs in the same molecule and can be recognized by different E3s: $S_{M,N,O...}$ for example, is targeted by $E2_B/E3_B$ and either $E2_C/E3_C$ or $E2_D/E3_C$ that recognize the two different sites, respectively. The model substrate lysozyme is targeted by the N-end rule E2–14kDa/E3α pair that recognizes the NH_2-terminal Lys residue. However, its complete degradation requires concomitant recognition by an additional pair of E2(s), probably a member(s) of the UBCH5 family of E2s, and an unidentified E3 that recognizes a downstream body motif (133).

4) $E3_D$ can interact with two different E2s, $E2_C$ and $E2_D$, to recognize and ubiquitinate a set of substrates ($S_{S,T,U...}$) via a common recognition motif. The F-box protein β-TrCP recognizes IκBα, HIV-1 Vpu, and β-catenin via a general doubly phosphorylated motif (see above). $SCF^{\beta\text{-TrCP}}$ acts both with members of the UBCH5 family of E2s as well as with the UBC3/Cdc34 E2s (131, 487). A different F-box protein, Skp2, which is the substrate-recognizing subunit of the SCF^{Skp2} E3 complex, targets the CDK inhibitor $p27^{Kip1}$ after a single phosphorylation on Thr-187 (52). It probably partners with the same E2s as $SCF^{\beta\text{-TrCP}}$.

5) $S_{P,Q,R...}$, like $S_{M,N,O...}$, is recognized by two distinct E3s, $E3_C$ and $E3_D$, each targeting a different motif. However, unlike $E3_B$ that is acting with a single E2 ($E2_B$), both $E3_C$ and $E3_D$ act each with two E2s, $E2_C$ and $E2_D$. p105, the precursor of the NF-κB transcription factor subunit p50, is targeted for limited processing by β-TrCP that recognizes a phosphorylated COOH-terminal motif similar to that of IκBα/β-catenin/HIV-1 Vpu (157, 158, 327). An additional, yet to be identified, E3 targets an acidic domain in the middle of the molecule (328). As described above, β-TrCP acts with members of the UBCH5 family and with UBC3/Cdc34 E2s, while the acidic site E3 acts probably with E2-25 kDa and members of the UBCH5 family of E2s (67) (A. Ciechanover, H. Gonen, and H. Achbert, unpublished data). The case of p105 and lysozyme may be similar to that of the *S. cerevisiae* MATα2 mating factor that has two degradation signals, Deg1 and Deg2. Deg1 is targeted by two E2s, Ubc6 and Ubc7 (58). Ubc4 and Ubc5 have also been implicated in targeting the mating factor, but it is not clear whether they are involved in recognition via the Deg2 motif. The E3 that acts along with the Ubc6/Ubc7 E2s has been identified as the ER/nuclear protein Doa10/Ssm4 (427a), but the ligase that acts along with Ubc4/Ubc5 has not been identified.

IV. MODES OF SUBSTRATE RECOGNITION AND REGULATION OF THE UBIQUITIN PATHWAY

Targeting of a protein via the ubiquitin system must involve specific binding of the protein to the appropriate ubiquitin ligase, E3. Despite recent progress in our understanding of modes of recognition and regulation of the system, it is only in a handful of cases where the E3 recognition motif has been identified precisely. In principle, recognition can be mediated via several mechanisms: either the substrate is modified so as to be recognized or not by the appropriate E3, or the activity of the E3 can be modulated. Although the number of cases is still too low to make sweeping generalizations, the mode of regulation appears to correlate with the class of E3. Ubiquitination by SCF complexes requires phosphorylation of the substrate; APC activity is modulated by the presence of ancillary substrate-binding factors and/or by phosphorylation of the complex subunits, and activity of the HECT-domain proteins may also depend on ancillary proteins that bridge between the enzyme and the substrate. E3α/Ubr1 appears to be constitutively active toward certain substrates and allosterically regulated toward others.

A. General Regulation

The ubiquitin-proteasome pathway can be regulated at the level of ubiquitination or at the level of proteasome activity. Because conjugation and proteasomal degradation is required for a multitude of cellular functions, regulation must be delicately and specifically tuned. In two cases however, it was reported that general rather than specific components of the pathway could be modulated by physiological signals. One is the upregulation of the pathway that is observed during massive degradation of skeletal muscle proteins. This occurs in mammals under normal fasting, but also under pathological conditions such as cancer cachexia, severe sepsis, metabolic acidosis, or after denervation (260, 308) (see sect. x*G*). It occurs also during specific developmental processes, such as insect metamorphosis; the massive breakdown of larval tissue before the development of the imago is accompanied by upregulation of the ubiquitin pathway (313). A different case in which degradation can be regulated is by changing the specificity of the proteasome cleavage sites within the frame of its function in antigen presentation (37, 124, 238, 370). It was shown that in mammalian cells, three components of the 20S proteasome, two that are encoded within the MHC locus and one that is encoded by a different region, are upregulated after interferon-γ treatment. They replace three other proteasomal subunits that confer to the proteasome a different peptide cleavage specificity, presumably favoring the types of peptides that are better bound to the major histocompatibility complex (MHC) class I molecules of the presenting cell and the T cell receptor of the cytotoxic T cell (CTL).

B. Specific Regulation

There are several modes for specific substrate recognition; some of them are depicted in Figure 3.

1. Recognition via the NH_2-terminal residue: the N-end rule pathway (Fig. 3A)

In recognition of substrates via the N-end rule pathway (454), substrates bind directly to the ligase via their NH_2-terminal residue. The E3 involved, E3α/Ubr1, has two N-end rule recognition sites: site I for substrates with basic NH_2 termini and site II for substrates with hydrophobic NH_2 termini (250, 368). A third site, site III, is involved in targeting non-N-end rule substrates. (see above and below). The targeting NH_2-terminal amino acids were designated "destabilizing" residues. Most of the studies on the N-end rule pathway were carried out using model substrates, and inactivation of several enzymatic components of the pathway did not yield a clear phenotype in yeast cells. Therefore, identification of substrates that are targeted via NH_2-terminal recognition has not been easy. RGS4, a member of the RGS (regulator of G protein signaling) involved in specific G protein activation, is arginylated posttranslationally at the NH_2 terminus, a modification that renders it sensitive to Ubr1-mediated ubiquitination and rapid degradation in vitro (73). However, because a substrate from which the N-end rule targeting motif was removed was still unstable in vivo, it is not clear whether this motif acts also in the context of the intact cell. Recently, Rao et al. (360) have shown that a 33-kDa fragment generated by separin (Esp1) from the cohesin subunit SCC1 during mitosis and chromatid sister separation contains an Arg residue at the NH_2 terminus and is degraded via the N-end rule pathway. Its degradation is essential for chromosome stability. It should be noted that activation of separin results from APC-mediated degradation of its inhibitor, Pds1 (securin). Activated separin then cleaves cohesin, the "glue" protein that attaches the two sister chromatids. This process is important for proper division of the genetic material between the daughter cells and its inheritance. The fragment is probably the first bona fide substrate of the N-end rule pathway.

2. Peptide binding-mediated allosteric activation (Fig. 3B)

Ubr1 has also a third recognition site that targets unidentified "body" motifs downstream from the NH_2-terminal residue (250, 368). Thus it can target *N*-α-acetylated proteins (132) and also the yeast Gα protein involved in growth regulation in response to pheromones (288, 387). The Cup9 transcription factor is a negative regulator of the di/tripeptide transporter Ptr1 gene. It is targeted by Ubr1, most probably after recognition by the body site (site III) of the enzyme. Peptides that bind to site I or II activate Ubr1 allosterically and increase the rate of catalysis of Cup9 ubiquitination and degradation. This in turn leads to induction of Ptr1 and to upregulation of peptides uptake, which reflects their increased concentration in the growth medium (447). Thus it is extremely important to discriminate between degradation following NH_2-terminal recognition of the substrate, a pathway that targets a limited subset of substrates, and recognition by E3α, the N-end rule pathway ligase that recognizes in addition many more substrates via non-N-end rule signals.

3. Phosphorylation of substrates and/or ubiquitination enzymes (Fig. 3, C–E)

Phosphorylation of substrates was shown to yield opposite effects on different substrates, and sometimes on different sites of the same substrate. An increasing number of substrates of the ubiquitin pathway are modified by phosphorylation before their ubiquitination, a modification that in certain cases at least was shown to be necessary for direct recognition of the modified protein by the appropriate E3. A nonexhaustive list includes the yeast G_1 cyclins Cln2 and Cln3, the yeast cyclin-dependent kinase (CDK) inhibitors Sic1 and Far1, the mammalian G_1 cyclins D and E, the mammalian CDK inhibitor $p27^{Kip1}$, the mammalian transcriptional regulators IκBα and β-catenin (244), and, recently, the NF-κB precursor p105 (157, 327). Strikingly, in all the instances where the E3 enzyme was identified, it turns out to be of the SCF type. Two of the best-characterized cases are those of IκBα and Sic1.

The mammalian transcription factor NF-κB is inhibited by IκBα, which binds to it and sequesters it in an inactive form in the cytosol. NF-κB is activated by a variety of extracellular stimuli such as cytokines, bacterial and viral products, and ionizing irradiation, among others (222). This activation is achieved by proteolysis of IκBα, which releases NF-κB to be translocated to the nucleus. Proteolysis of IκBα requires its phosphorylation on two specific residues, Ser-32 and Ser-36, after which it is recognized by a specific SCF complex, $SCF^{\beta\text{-TrCP}}$ (487). Activity of $SCF^{\beta\text{-TrCP}}$ appears to be constitutive, while the IκBα kinases, IKKα and IKKβ, are activated by the NF-κB-inducing stimuli. Another substrate of $SCF^{\beta\text{-TrCP}}$, β-catenin, is phosphorylated by a distinct kinase, GSK3β, on two serines embedded in a sequence similar to the Ser-32-Ser-36 region of IκBα (151). These two regions together with a third β-TrCP-binding protein, HIV-1 Vpu (293), allow the definition of a consensus recognition motif for β-TrCP binding: DS(P)GψXS(P) [single-letter amino acid code; S(P) stands for phospho-serine, ψ stands for a hydrophobic residue]. This sequence by itself is sufficient for binding of the ligase, since a phosphopeptide that spans this recognition region can immobilize β-TrCP and inhibit IκBα ubiquitination and degradation both in vitro and in vivo (486). A similar motif has recently been described for the NF-κB precursor protein p105 (157, 327). After IKK-mediated phosphorylation, the target proteins are ubiquitinated by $SCF^{\beta\text{-TrCP}}$. Unlike β-TrCP, however, no consensus recognition motif has been defined yet for other F-box proteins.

Sic1 is an inhibitor of yeast B-type (Clb) cyclin/CDK complexes, but not of G_1 (Cln) cyclin/CDK complexes. Rapid degradation of Sic1 at the end of G_1 enables the initiation of DNA replication (398). In wild-type cells, Sic1 appears at the end of mitosis and disappears shortly before S phase. G_1 cyclin/CDK complexes phosphorylate Sic1 at a number of specific sites (455); the phosphorylated protein is then recognized by the SCF^{Cdc4} ubiquitination complex and degraded (398, 455). Interestingly, and unlike recognition of defined phosphorylated residues by TrCP, the affinity of CDC4 to the phosphorylated Sic1 increases with the number of phosphorylated residues (317a). All available evidence indicates that Sic1 degradation is uniquely regulated by its phosphorylation, while the SCF^{Cdc4} activity is constitutive: the cell cycle-specific degradation is due to the temporal expression of the G_1 cyclins that are required for Sic1 phosphorylation, and when these G_1 cyclins are ectopically expressed, Sic1 becomes constitutively unstable (21). Further support for the assumption that SCF^{Cdc4} activity is constitutive comes from the observation that Gcn4, another SCF^{CDC4} substrate, is constitutively degraded during the cell cycle (244, 304).

Degradation of the mammalian G1 CDK inhibitor $p27^{Kip1}$ is required for the cellular transition from quiescence to the proliferative state. Ubiquitination and subsequent degradation of p27 depend on its phosphorylation by G_1 cyclin/CDK complex at a specific residue, Thr-187, which is followed by SCF^{Skp2}-mediated ubiquitination (52, 445). Interestingly, both in vivo and in vitro, Skp2 is a rate-limiting component of the machinery that ubiquitinates and degrades phosphorylated p27; its expression is cell cycle regulated and peaks at the S phase (496). Thus p27 degradation is subject to dual control by the presence or absence of the specific F-box protein and of cyclin A/CDK complexes that are regulated by mitogenic stimuli. The transcription factor E2F-1 is also ubiquitinated by SCF^{Skp2} (294); however, it is not known yet whether E2F-1 degradation also requires phosphorylation. Interestingly, it has been reported that many short-lived proteins contain a sequence enriched in PEST residues (Pro, Glu, Ser, Thr) (372) that has been implicated in destabilization of the proteins. Although the role of the region has never been deciphered, it appears to act via phosphorylation of its Ser/Thr residues.

Experiments with APC/cyclosome from clam oocytes demonstrated that enzyme isolated from interphase extract could be activated by the Cdc2 kinase, and conversely, enzyme isolated from mitotic extracts could be inactivated by the addition of phosphatase (252). Although it was suggested that Cdc2 can directly phosphorylate certain APC/cyclosome subunits, the kinetics of APC/cyclosome activation suggest that the enzyme subunits may not be directly activated by Cdc2 and that the kinase acts upstream to activate the ligase via modification of intermediary kinases (333). Immunoprecipates of Plk, a protein kinase related to the *D. melanogaster* POLO, revealed that the protein is complexed to APC1, Cdc16, and Cdc27. Furthermore, Plk was shown to be activated by Cdc2 (245). Purification of *Xenopus* and human APC/cyclosomes revealed that at least four subunits, APC1, CDC16, CDC23, and CDC27, are hyperphosphorylated in mitotic extracts (344). While the physiological role of subunit phosphorylation in APC is currently unclear, it appears that modification of a certain subunit(s) by specific kinase may affect its activity against a certain subset of substrates at a particular point of mitosis (see also below).

Subunit phosphorylation also appears to negatively regulate APC/cyclosome activity. Experiments in *S. pombe* implicate the protein kinase A (PKA) pathway as an inhibitor of APC activity. For example, addition of mammalian PKA to cyclosome fractions inhibits cyclin B ubiquitination even if the ligase was previously activated by Plk (245). Overexpression of cAMP phosphodiesterase in *cut4–533*, a mutant defective in APC/cyclosome complex formation, restores incorporation of the ligase subunits into high-molecular-mass complex, suggesting that subunit phosphorylation by PKA may negatively regulate the assembly process (483). Phosphatases have also been implicated in APC/cyclosome activation. Type I protein phosphatases (PP1) are required for the onset of anaphase in many eukaryotes (171), and an APC6 (*cut9–665*) mutant is synthetically lethal with mutations in *dis2+* that encodes a catalytic subunit of PP1 (481). Thus it appears that the APC/cyclosome is positively and negatively regulated by an intricate network of kinases and phosphatases, and each acts probably on a specific subunit(s) and modulates the activity of the enzyme toward a specific substrate and at a specific point along the mitotic process.

Targeting of the epidermal growth factor receptor (EGF-R) involves initial autophosphorylation at a previously identified lysosome-targeting motif that leads to recruitment of the RING finger ligase c-Cbl/Sli-1. This is followed by Tyr phosphorylation of the ligase at a site adjacent to the RING finger domain that allows receptor ubiquitination and subsequent routing to the lysosome for degradation (269).

Phosphorylation can also inhibit ubiquitination as has been described in several instances. Degradation of the protooncogene c-*mos* by the ubiquitin pathway is inhibited by phosphorylation on Ser-3 (319, 320). Interestingly, activation of c-*mos* leads to phosphorylation and stabilization of c-*fos*, another substrate of the ubiquitin pathway. Although c-*mos* is a serine/threonine kinase, it probably does not phosphorylate c-*fos* directly. Rather, it is thought to act via activation of the MEK1/ERK pathway (325). c-Jun also appears to be stabilized by phosphorylation after its association with the signalosome complex (see below) (318, 401). Another example is that of the anti-apoptotic protein Bcl-2: dephosphorylation of Bcl-2 upon apoptotic stim-

uli renders it susceptible to degradation by the ubiquitin pathway (89).

4. Recognition in trans *(Fig. 3F)*

In several cases, the target protein is not recognized directly by the ligase, but rather in *trans*, following binding to an ancillary protein. Viruses were found to exploit the ubiquitin system by targeting cellular substrates that may interfere with propagation of the virus. In some instances, the viral protein functions as a bridging protein between the E3 and the substrate. The prototype of such an interaction is the HECT domain ligase E6-AP that acts along with the HPV protein E6 to target p53. E6 binds both p53 and E6-AP, and formation of this ternary complex results in the ubiquitination and degradation of p53. This activity of E6 can account, at least partially, for the oncogenicity of the strains of papillomaviruses that express it (388). A second instance of a viral protein-mediated ubiquitination of an endogenous substrate is that of the degradation of the T-cell CD4 receptor. As mentioned above, the Vpu protein of the HIV-1 virus is recognized, after phosphorylation, by the F-box protein β-TrCP. Vpu also binds to the CD4 receptor of the T cells infected by the virus; this binding leads to ubiquitination and degradation of CD4 by the $SCF^{\beta\text{-}TrCP}$ complex (293). This case is interesting in particular as the phosphorylated protein Vpu to which the E3 binds is not ubiquitinated and remains stable. It is the CD4 protein that is not phosphorylated that is targeted for degradation. The problem is obviously how a substrate to which the ligase is not bound is targeted *in trans*. In a different case, molecular chaperones appear to facilitate ubiquitination and degradation of certain proteins. This has been shown for several soluble model proteins (29, 262), and not surprisingly also for membrane proteins. Thus the chaperone BiP was demonstrated recently to bind to a mutant Prion protein and to mediate its degradation by the proteasome (205). Similarly, mutated CFTR and the glucocorticoid receptor are degraded after complex formation with the cochaperone and E3 CHIP and the chaperones Hsc70 and Hsc90, respectively (see above).

5. Abnormal/mutated/misfolded proteins (Fig. 3G)

The ubiquitin system selectively and efficiently degrades denatured/misfolded proteins that arise as a result of mutations, immaturation, or posttranslational environmental stress (62, 162, 377, 403). It can also degrade cotranslationally nascent peptide chains that do not attain native structure due to errors in translation or in posttranslational processes necessary for proper folding. Cotranslational degradation is an extensive process, and ~30% of nascent chains are degraded and do not mature to native proteins (393, 448). The degraded chains were designated DRiPs (defective ribosomal products). It should be noted, however, that these products have not been shown to be defective though premature/incomplete polypeptide chains (such as are generated in the presence of puromycin) are known to be preferred substrates for the ubiquitin system. The nature of the signals and the identity of the E3(s) involved are still mysterious. It is possible that exposure of hydrophobic domains that are normally buried in protein-protein interaction surfaces or within the protein core, serve as recognition signals for immature, nascent, and otherwise abnormal and misfolded proteins. It is possible that since nascent chains associate with chaperones prior to completion of synthesis of the entire polypeptide chain, the E3 CHIP may be involved in their targeting (see above). An interesting case involves the oxygen-induced ubiquitination of HIF-α by the pVHL ligase complex, where the ligase recognizes specifically a hydroxylated Pro residue (Pro564) generated in the target protein under hyperoxia. In this case, however, although the modification renders the protein "abnormal" by generating an oxidized amino acid derivative, it is a specific and regulated change, catalyzed by a specific enzyme, prolyl hydroxylase (47a), that targets for degradation, under high oxygen pressure, a transcription factor that operates normally during normoxia or hypoxia (see above).

6. Recognition via specific sequences (not shown in Fig. 3)

Ubiquitination of mitotic cyclins is mediated by a small NH_2-terminal motif known as the "destruction box." The minimal motif is nine residues long, and it has the following consensus sequence: **R**-A/T-A-**L**-G-X-I/V-G/T-N (absolutely conserved residues are in boldface) (482). The function of the destruction box is not clear, because it is not phosphorylated or ubiquitinated. It may serve, however, as a binding site for the ligase subunit of the APC/cyclosome complex. Destruction boxes may not be the only determinants of cyclin stability. The boxes of cyclin A and B are not interchangeable, and indestructible cyclin constructs that contain a destruction box derived from a different cyclin can still be polyubiquitinated (239). Thus, although ubiquitination is probably necessary for cyclin destruction, it may not be sufficient.

7. Regulation by ubiquitin-like proteins (not shown in Fig. 3)

Both enzymes and substrates of the ubiquitin system have been found to be modified by ubiquitin-like proteins (UBL; see below). In the case of enzymes, it affects their activity or stability. In the case of substrates, it affects their availability to the ubiquitination/degradation machinery.

The UBL NEDD8 was shown to modify each of the five mammalian Cullins, Cul1–5. The unique yeast Cullin, Cdc53, is modified by Rub1, the yeast homolog of NEDD8 (488). NEDD8 conjugation to the Cul1 component of SCF^{Skp2} was

shown to be important for the activity of the enzyme in catalyzing ubiquitination of the CDK inhibitor $p27^{Kip1}$ (354). The mechanism(s) that underlie the NEDD8 conjugation-induced activation of the SCF^{Skp2} complex is still obscure but it may be important for recruitment of the E2 enzyme (223a). Modification of Mdm2 by SUMO-1 abrogates its self-ubiquitination and increases its ligase activity toward p53. Radiation-induced DNA damage caused a gradual decrease in SUMO-1 modification of Mdm2, which led to its destabilization with subsequent increase in p53 level (49). Here, it appears that SUMO modification blocks ubiquitination and thus contributes to the stability of the enzyme. It was suggested that while SUMO modification directs the ligase activity toward its native external substrate, desumoylation directs Mdm2 self-ubiquitination. It was further suggested that it is the modification-demodification cycle that is actually regulated by the irradiation/DNA damage. A similar case was described for IκBα, where SUMO modification of Lys-21, one of the two Lys residues that serve as anchors for the ubiquitin chain, stabilizes the protein not only by inhibiting ubiquitination, but also by inhibiting phosphorylation on Ser residues 32 and 36, a modification that precedes ubiquitination (83). It should be noted that in this case, unlike the modification of Mdm2, the physiological relevance of the process is yet to be revealed. The experiments were carried out in a system that overexpresses SUMO-1, and it is not clear that even under these conditions, sumoylation involved all the cellular pool of the substrate, as physiologically, cytokine-induced phosphorylation and ubiquitination leads to a rapid and complete disappearance of the entire cellular pool of the inhibitor.

8. Regulation by masking of a degradation signal (not shown in Fig. 3)

In yeast, the mating type of haploid cells is determined by the presence of either one of two transcription factors, **a**1 and α2. When two haploid cells of opposite types mate, the resulting diploid expresses both **a**1 and α2, which form a heterodimer with a distinct DNA binding specificity. In haploid cells, the **a**1 and α2 proteins are rapidly degraded by the ubiquitin system, probably to enable the cells to efficiently switch mating types. The degradation of α2 (MATα2) has been studied in detail. One of the two degradation signals, Deg1, contains residues on the hydrophobic face of a predicted ampiphathic α-helix. Strikingly, both **a**1 and α2 are stabilized by heterodimerization. At least for α2, it has been shown that residues that are important for the interaction with **a**1 overlap with the Deg1 signal (211). The simplest interpretation of these results is that binding of **a**1 interferes with the recognition of α2 by the ubiquitin system because of masking of the degradation signal. In a different case that also involves a developmental program, it was recently shown that the *Drosophila* homeobox protein *Homothorax* is stabilized in cells expressing its binding partner *Extradenticle* (3). In this case however, the underlying mechanisms have not been dissected, although the analogy to the yeast case is attractive. It should be noted that exposure of hydrophobic domains that are normally hidden within the core of the native protein or within protein-protein interaction surfaces may trigger preferential ubiquitination (see above). Another example of protection by association involves the interferon regulatory factors (IRFs). IRFs are a family of nine transcription factors that are recruited upon interferon signaling to activate target genes involved in combating viral infection. Some of these genes code for certain catalytic β_i-subunits of the 20S core particle of the proteasome (CP; see sect. VI) (106, 290). IRF-1 (as well as some other members of the IRF family) is normally an unstable protein and a substrate for the proteasome. Upon phosphorylation, IRF-8 heterodimerizes with IRF-1 and protects it from degradation (64). Interestingly, the COOH-terminal domain of IRF-1 that controls its stability is also the region to which IRF-8 binds (316). Thus it is possible that the complex of IRF-8 with IRF-1 is protected from degradation by the proteasome since the same module in IRF-1 is essential both for its destabilization and for IRF-8 binding.

In two different instances, it was found that a DNA binding protein could be stabilized by binding to its cognate DNA. The myogenic transcription factor MyoD can be efficiently ubiquitinated in vitro; however, binding of MyoD to its specific cognate DNA sequence protects it from ubiquitin-mediated degradation (2). Similarly, E6-mediated degradation of p53 is also inhibited by binding of the tumor suppressor to its cognate DNA (310).

9. Regulation by specificity factors (not shown in Fig. 3)

Regulated degradation of specific classes of substrates could be achieved by modulation of the activity of the ubiquitination machinery. This can be attained by association of E2/E3 complexes with different ancillary factors. Recent data regarding degradation of mitotic substrates place the APC within that category. The paradox of APC activity during the different phases of mitosis was due to the fact that it was isolated biochemically, and its components were identified genetically as being required for the degradation of B cyclins (193, 228). However, B cyclins are degraded at the end of mitosis, whereas the APC is required also, both in yeast and in mammalian cells, much earlier, for onset of anaphase (193, 446). This paradox was resolved by the isolation of APC substrates that act as inhibitors of metaphase to anaphase transition, Cut2 (in *S. pombe*) and Pds1 (in *S. cerevisiae*) (65, 113). Cut2 and Pds1 are degraded at the beginning of anaphase. The enigma of how the degradation of the various APC substrates is differentially and temporally regulated has been resolved by the isolation of proteins that are not part

of the core of the APC, but bind to it substoichiometrically and confer to it different substrate specificities. These are members of the WD-40 repeat protein family, Cdc20/Fizzy and Hct1/Cdh1/Fizzy-related. Overexpression of Cdc20p/Fizzy causes ectopic APC-mediated destruction of Pds1 under conditions when the protein should be stable (hydroxyurea-treated, S phase-arrested cells, or nocodazole-treated, early M phase-arrested cells; Ref. 457). In contrast, binding of Hct1/Cdh1/Fizzy-related is required for degradation of cyclin B as well as other late mitotic substrates such as Ase1 (395). Activities of both Fizzy and Fizzy-related are regulated. Fizzy appears to be the target of a mitotic checkpoint: failure of spindle assembly leads to binding of the checkpoint proteins Mad1–3 to Cdc20, resulting in inhibition of mitosis (100, 192). Regulation of Fizzy-related is achieved by CDK-mediated phosphorylation: phosphorylated Fizzy-related is unable to bind to the APC and to activate it (200, 494). Interestingly, the Cdh1/Fizzy-related-associated APC recognizes a unique signal in the target substrate, a K-E-N box. Cdc20/Fizzy is itself a Cdh1-APC substrate. Vertebrate Cdc20 lacks the D box and is recognized by Cdh1-APC through a different sequence composed of K-E-N that serves as a general targeting signal for Cdh1-APC. Like the D box, it can confer proteolytic sensitivity to other proteins. Other cell cycle related gene products such as Nek2 and B99 have been also shown to be KEN-box containing, Cdh1-APC substrates. Mutation in the KEN box stabilizes all three proteins against ubiquitination and degradation (346). Recent evidence indicates that these two proteins are the specific substrate-binding subunits of the APC during the different phases of mitosis. Thus Hct1/Cdh1 recognizes and binds the mitotic cyclin Clb2 and also Clb3 and Cdc5, while Cdc20 binds Pds1 and targets them for ubiquitination by the APC (395a).

V. "NONCANONICAL" PATHWAYS OF THE UBIQUITIN SYSTEM

For many years, the accepted model for ubiquitination had been an E1/E2/E3-catalyzed cascade reaction in which the first ubiquitin moiety is anchored via its COOH-terminal Gly residue to an ϵ-NH_2 group of an internal Lys residue in the target substrate. This is followed by generation of a polyubiquitin chain in which additional ubiquitin moieties are linked to one another via a Gly-76-Lys-48 isopeptide bonds. During the years, many "exceptions" to this "rule" have been discovered that can be classified into two groups: *1*) reactions catalyzed by a different set of enzymes and *2*) reactions that utilize different anchoring sites whether on the substrate or on the ubiquitin moiety.

A. Reactions Catalyzed by Noncanonical Enzymes

Four distinct modes of conjugation are catalyzed by different enzymes (Fig. 4).

1) UBL proteins (for their role, see below) are generally, but not always, activated by an activating enzyme, "E1," that is a heterodimer. One subunit (E1a) bears homology to the NH_2-terminal domain of E1, while the other, E1b, resembles the COOH-terminal domain of E1. NEDD8 (Rub1; yeast proteins in the following section are in parentheses), for example, is activated by APP-BP1 (Ula1-Uba3 in yeast) (134, 273). Another UBL protein, SUMO-1

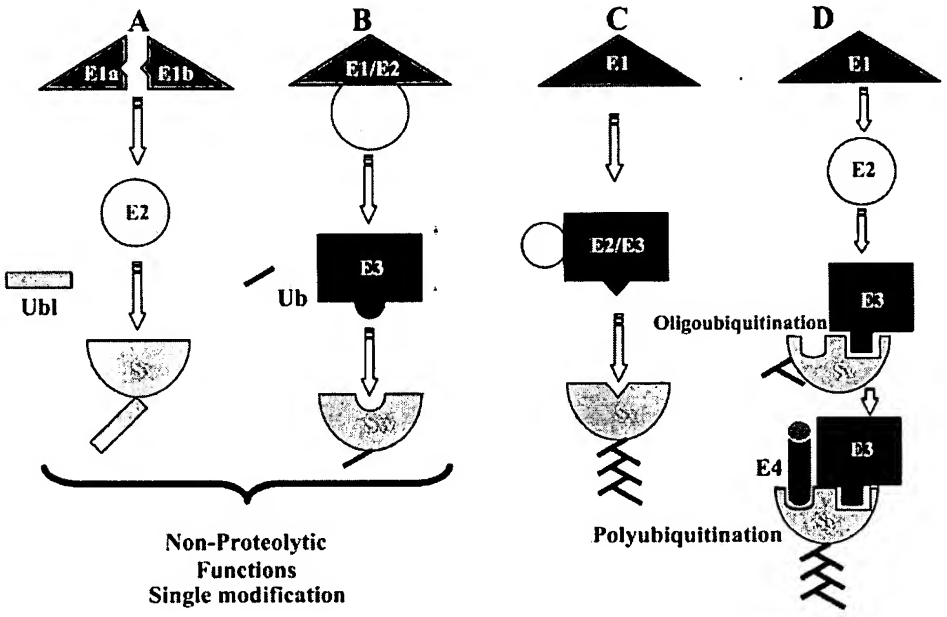

FIG. 4. Noncanonical pathways for conjugation of ubiquitin or ubiquitin-like proteins (UBLs). Color code is as in Figure 2. *A*: activation of UBL. *B*: activation of ubiquitin by a hybrid E1-E2 enzyme. *C*: activation of ubiquitin by an E2-E3 hybrid enzyme. *D*: E4 is involved in elongation of short ubiquitin chains. For details, see section V.

(Smt3), is activated by UBA2-AOS1 (same terminology for the yeast and mammalian enzymes) (208). UBLs also have unique conjugating "E2" enzymes. The E2 involved in NEDD8 (Rub1) activation is Ubc12 (134), whereas the E2 involved in SUMO-1 (Smt3p) activation is Ubc9 (210). Activation and conjugation of an additional small protein modifier, Apg12 involved in autophagy in yeast, is mediated by a single subunit E1, Apg7, and a single subunit E2, Apg10 (309).

Even though the activation reactions of ubiquitin and UBLs are similar, the E1 and E2s involved in the two processes cannot replace one another. This is true even for activation of the different UBLs. This lack of cross-activation is important to ensure tight regulation of the distinct processes regulated by the two systems. Another point of difference from the ubiquitination machinery is that the substrate recognition components have not been discovered. While initial findings have not shown a role for an E3 in the process (173), it is not clear that such components do not play a role in modification of proteins by UBLs. Thus the substrate recognition function could be carried out by the E2 or the E1/E2 complex directly, but also by a specific "E3" for UBL proteins (see for example Ref. 208a).

2) In a different case, ubiquitin is activated by a hybrid activating-conjugating, E1-E2, enzyme. $TAF_{II}250$ in *Drosophila melanogaster* is a histone-specific ubiquitin activating/conjugating (E1-E2) enzyme (UBAC) involved in monoubiquitination of histone H1. Its inactivation results in a lower expression of mesoderm-determining genes regulated by the maternal activator Dorsal (347). An E3 does not appear to be involved in this reaction.

3) E2–230 kDa is an exceptionally large E2. Mechanistic analysis using differential inhibition of two active cysteine residues suggests that the enzyme functions as an E2-E3 hybrid (31). While the enzyme appears to catalyze polyubiquitination, the identity of the native substrates of this enzyme is yet unknown.

4) In yeast, UFD2 is a U box-containing enzyme that functions as an E4 involved in elongation of short ubiquitin chains, generated most probably via E1/E2/E3 catalysis (241). This E4 is part of the UFD pathway (see above). The native substrates of this pathway have not been identified yet. It is not clear why E4 is necessary for elongation that cannot be catalyzed, as in the case of the substrate studied, by E3 alone, since for most cases, E3 is sufficient to generate the polyubiquitin chain on the substrate with no need for additional ubiquitinating enzymes (i.e., E4) (see sect. II*C*). Other mammalian U-box proteins such as CHIP (see above) have been shown to conjugate ubiquitin molecules without the need for an additional E3 (153; see also above). It is possible that in certain cases E3 catalyzes monoubiquitination or oligoubiquitination in a defined subcellular localization, while generation of the polyubiquitin chain is catalyzed by E4 in a different site. This can be the case, for example, for p53 for which it has been shown that Mdm2 mediates modification by single ubiquitin moieties, each on a distinct lysine residue (253).

B. Alternatively Assembled Polyubiquitin Chains

The second group of noncanonical conjugation reactions involves anchoring of ubiquitin to *1*) sites other than an internal Lys residue of the target substrate or *2*) Lys residues other than Lys-48 of the previously conjugated ubiquitin moiety. The different modes of ubiquitination and their physiological relevance were reviewed recently (349, 350).

1) As described above, certain substrates, MyoD, LMP1, and HPV E7, for example, are targeted by fusion of the first ubiquitin moiety to their NH_2-terminal residue rather than to an internal lysine.

2) Ubiquitination on Lys-63 of ubiquitin appears to play a role in a variety of processes, including endocytosis of cell surface receptors (166, 426), postreplicative DNA repair (418), stress response (16), mitochondrial DNA inheritance (104), ribosomal function (417), and activation of the IκBα signaling complex (79, 461) (see above). It appears that conjugation in position 63 requires distinct conjugation enzymes. In the case of the IκBα signaling complex, a hybrid E2 is generated between the UEV Mms2 and Ubc13 (79, 179) (see above). Indirect experimental evidence, however, suggests that this type of modification does not involve proteolysis of the target substrates, but rather the modification plays a role in the activation/inactivation of the target protein.

3) Another mode of conjugation involves linking ubiquitin molecules via Lys-29 of ubiquitin (295, 491). At least in one case it was shown that short Lys-29 chains are involved in recruitment of the chain-elongating factor E4 (241).

4) Enzymes that can catalyze formation of multiubiquitin chains linked via lysine-6, or lysine-11, are also known; these chains can bind to the proteasomal subunit Rpn10/S5a, although it is not clear if they actually target substrates for degradation (19).

5) In several cases it has been reported that modification by a single ubiquitin moiety (monoubiquitination) is sufficient to trigger the process in which the ubiquitination is involved. This has been described in yeast, for example, for endocytosis of the α-factor receptor and the stable (model) membrane protein Pma1 (410) (see below). An interesting case involves Fanconi anemia (FA). FA is a human autosomal recessive cancer susceptibility disorder characterized by cellular sensitivity to mitogens and ionizing radiation. While six FA genes (A, C, D2, E, F, and G) have been cloned, their relationship to DNA repair has remained unknown. It has been recently shown that a nuclear complex containing the FANCA, FANCC, FANCF, and FANCG proteins is required for the activation of the FANCD2 protein to a

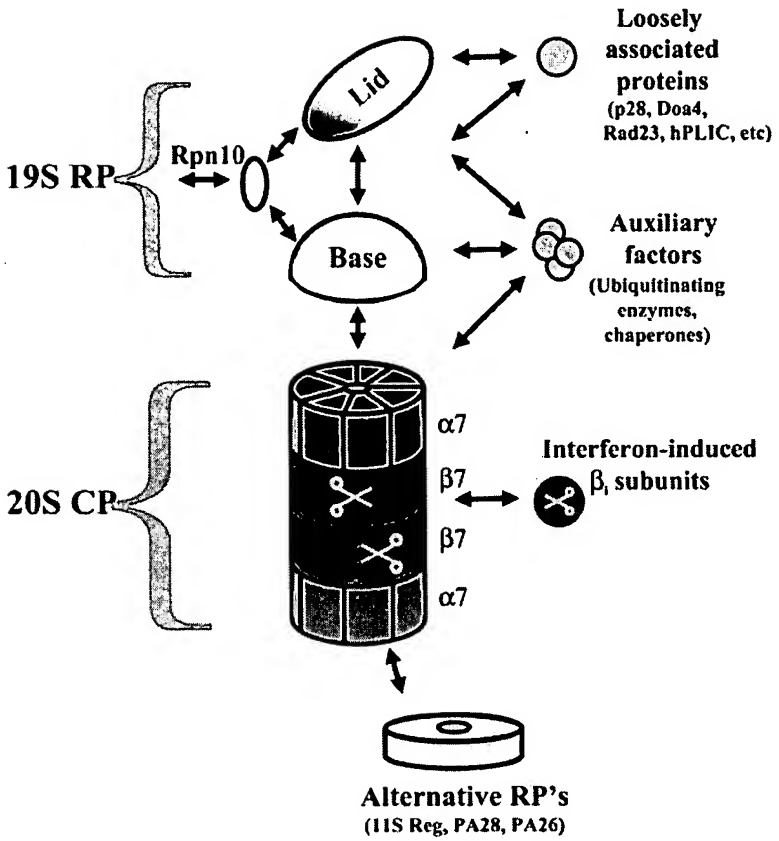

FIG. 5. Structure of the proteasome. The proteasome is a modular structure. One or two regulatory particles (RP) attach to the outer surface of the core particle (CP). The CP is made up of four heptameric rings: two outer identical α-rings and two identical inner β-rings. Each ring is made of seven distinct homologous subunits. Certain β-subunits contain the protease active sites facing inward into the sequestered proteolytic chamber. Upon interferon-γ induction, three β-subunits can be replaced by βi (LMP) homologs that alter the proteolytic specificities of the proteasome. The 19S RP is comprised of two eight-subunit subcomplexes, the lid, and the base. The base that contains all six proteasomal ATPases attaches to the α-ring of the CP. The lid can disassociate from the proteasome, resulting in a truncated base-CP complex. Rpn10 can interact with either the lid or the base and stabilizes the interaction between the two. Rpn10 is also found outside of the proteasome. Numerous associated proteins and auxiliary factors, such as chaperones and components of the ubiquitination machinery, can interact with the RP. Alternative regulatory complexes can also attach to the surface of the α-ring.

monoubiquitinated isoform. In normal cells, FANCD2 is monoubiquitinated in response to DNA damage and is targeted to nuclear foci where it is colocalized with the breast cancer susceptibility protein, BRCA1, where they probably function together in DNA repair. Disruption of this pathway may result in the cellular and clinical phenotype common to all FA subtypes (118).

VI. THE PROTEASOME AND DEGRADATION OF UBIQUITINATED SUBSTRATES

A. Structure of the Proteasome

1. 26S proteasome

The proteasome holoenzyme (also known as the 26S proteasome) is a ~2.5 MDa complex made up of two copies each of at least 32 different subunits that are highly conserved among all eukaryotes (Fig. 5). The overall structure can be divided into two major subcomplexes: the 20S CP that contains the protease subunits and the 19S regulatory particle (RP) that regulates the function of the former (Fig. 5). The 20S CP is a barrel-shaped structure made up of four rings of seven subunits each. The two inner β-rings contain the proteolytic active sites facing inward into a sequestered proteolytic chamber (143, 278). One or two regulatory particles attach to the surface of the outer α-rings of the 20S CP to form the 26S proteasome holoenzyme (Fig. 5). The 19S RP itself can be further dissected into two multisubunit substructures, a lid and a base (126).

Six homologous ATPases (Rpt1–6) are present in the base together with three non-ATPases subunits (Rpn1, -2, and -10). Direct interactions have been reported between these ATPases and the α-subunits of the CP (72a, 109a). The ATPases in the base most likely unfold substrates and translocate them through a gated channel into the 20S CP (44, 143, 183, 242, 425). The lid of the RP is a 400-kDa complex made up of eight of the remaining non-ATPase subunits (Rpn3, -5, -6, -7, -8, -9, -11, -12) that can be released from the proteasome or rebind under certain conditions. The role of the lid is still unclear, although it is necessary for proper degradation of polyubiquitinated proteins (126). All subunits of the lid subcomplex contain one of two structural motifs: Six subunits contain a PCI domain (proteasome, COP9, eIF3), while the other two (Rpn8 and Rpn11) contain an MPN domain (Mpr1, Pad1 NH_2-terminal) (13, 126, 177). The lid shares significant genetic and structural features with other cellular complexes, indicating that there might be structure-function relationships common to a number of different regulatory pathways.

The structure and subunit composition of proteasomes purified from different species or by different protocols are almost identical (76, 91, 127, 174, 455a). In a few preparations, a unique subunit was identified that is not present in genomes of other species or that has not been identified in other preparations (for example, Son1/Ufd5/Rpn4 in *S. cerevisiae*, S5b in human, and p37a in *Drosophila*); these factors could be transiently associated, substoichiometric, or species specific (84, 112, 174).

Development-dependent changes account for some of the diversity in proteasome composition. Higher levels of proteasome and 19S RP components are found in stationary yeast compared with rapidly growing yeast (111). The growing-to-stationary phase transition also induces the assembly of proteasome holoenzymes from its 20S CP and 19S RP subcomplexes, probably favoring doubly capped (RP_2CP) proteasomes over singly capped (RP_1CP) or free CP (111). A similar observation was made for elevated proteasome levels in *Manduca sexta* during cell death (74, 75). Most likely, basal expression levels of the principal 31 proteasomal genes are quite similar based on the seemingly stoichiometric levels of proteasomal subunits in purified samples. Likewise, almost all 20S CP and 19S RP genes contain an identical PACE element (the nonamer sequence 5′-GGTGGCAAA-3′) in their promoter situated at the same distance upstream from the start codon, indicating that they might be under coordinate transcriptional control (292). A notable exception is Rpn10, which does not contain this element and is the only proteasomal gene found in significant levels unbound to the proteasome (150, 453).

Proteolysis of short-lived or regulatory proteins, whether from the cytoplasm, the nucleus, or the ER, is carried out predominantly by the proteasome. All but two of the genes encoding 19S RP subunits in budding yeast are essential, and deletions of all but one of the 20S CP subunits are lethal (125, 127, 128). In some instances, single amino acid substitutions in a single proteasomal subunit cause lethality in yeast, while other mutants cause general proteolysis defects, and sensitivity to stress conditions that increase the levels of damaged proteins (such as elevated temperatures, or exposure to amino acid analogs) (377). In mammalian systems, there have been some reports that cells can adapt under exposure to a proteasome inhibitor (345, 462). It was suggested that elevated levels of a large ATP-independent protease, tripeptidyl peptidase II (TPPII), was responsible for viability in these proteasome-comprised cells (345, 462). However, it was later found that residual proteasome activity was present in these cells and that viability depends on functional proteasome, indicating that TPPII cannot replace essential proteasome activities (356). Therefore, the proteasome is probably an essential component of all eukaryotic cells. In general, the ubiquitin/proteasome pathway is probably one of the most, if not the most, conserved regulatory pathways in eukaryotes. Most proteasome subunits exhibit >40% identity between yeast and humans, and some key components (such as the six proteasomal ATPases) maintain 70–80% identity between yeast and humans. The great majority of subunits are conserved functionally, with human homologs rescuing knockouts of yeast proteasomal genes (125, 128).

It is likely that the proteasome is a modular system that was refined over the course of evolution and adapted to its indispensable regulatory roles in eukaryotes. For instance, the eukaryotic proteasome has been adapted to degrading proteins in a ubiquitin-dependent fashion by the addition of regulatory factors that assemble in different layers onto the ancient proteolytic core of the proteasome, as well as by increasing the diversity of the principal subunits themselves.

2. 20S CP

The proteolytic activity of the proteasome is found within the 20S CP (37, 156). Although purified free 20S CP can hydrolyze small peptides and some unfolded proteins, it cannot degrade multi-ubiquitinated proteins. The structure of 20S CP purified from yeast was determined by X-ray crystallography (143). The CP is a hollow cylindrical structure composed of four heptagonal rings, stacked in C_2 symmetry (Fig. 5). Each of the two outer rings is comprised of seven genetically related and structurally similar α-subunits, and each of the two inner rings is comprised of similarly conserved β-subunits; thus the CP barrel as a whole also exhibits pseudo-sevenfold symmetry (in addition to the C_2 symmetry). In eukaryotes, three of the seven β-subunits have functional threonine protease active sites, meaning that each proteasome has six (3 different) proteolytic active sites. In archaea, all β-subunits are identical, leading to 14 active sites in total. The protease active sites face an inner cavity within the β-rings that can be accessed through a narrow channel leading from the surface of the α-rings (142, 242). Upon interferon-γ signaling, a change occurs in proteasome subunit composition by replacement of the proteolytically active β-subunits with alternative, so-called LMP or βi, subunits. Each of these βi-subunits is genetically homologous to a specific constitutively expressed β-subunit and can be incorporated into the corresponding position within the β-ring of newly assembled proteasomes. β1i/LMP2 replaces its constitutively expressed β1-homolog, β5i/LMP7 replaces β5, and β2i/LMP10 replaces β2 (37). The subunit composition of proteasomal core particles is thus altered upon γ-interferon induction, and they are therefore often referred to as "immunoproteasomes." Immunoproteasomes are thought to generate peptides that are more appropriate for antigen presentation (238, 489).

The NH_2 termini of the α-subunits obstruct access to the proteolytic chamber, suggesting that the proteasome channel is gated (142, 143). The NH_2 terminus of α3 is somewhat distinct from the other α-subunits in that it

points directly across the α-ring surface toward the center of pseudo-sevenfold symmetry with close contacts to every other α-subunit. For substrates to enter the CP, and most likely for products to exit as well, the blocking NH_2-terminal residues of these α-subunits must be moved and rearranged. Interestingly, the formation of a well-defined closed configuration of the gate involves a dramatic departure from the pseudo-sevenfold symmetry of the CP. Even though the seven α-subunits are genetically related (~30% identical to each other in yeast) and structurally almost superimposable (143), their NH_2-terminal tails are different from each other both in sequence and relative length (142). At the same time, these NH_2-terminal tails are absolutely conserved among orthologs from numerous species, suggesting that the NH_2 termini play a critical structural role that has been maintained in core particles in all eukaryotes, and it is precisely the difference between them that is integral to their function.

The peptidase activity of the eukaryotic 20S CP can be stimulated by a variety of treatments. For instance, stimulation occurs when the CP complexes with the RP to form the 26S proteasome holoenzyme (4, 127). Other endogenous activators of the CP include the interferon-induced PA28/11S REG complex (364, 423) or a distantly related activator, PA26 (469, 485). Mild chemical treatments, such as exposure to low levels of sodium dodecyl sulfate (SDS), are also effective (127). A nine-residue deletion mutation of the NH_2-terminal tail of the α3 subunit (α3ΔN) results in constitutively activated peptide hydrolysis by the CP (142). While core particles purified from wild-type yeast can be stimulated by additions of small amounts of SDS, core particles purified from the α3ΔN mutant strain are constitutively and maximally stimulated. This result indicates that the wild-type free CP is found in a repressed state, and activation possibly reflects facilitated substrate access into the proteolytic chamber. Thus the NH_2 termini of the α-subunits in wild-type CP appear to regulate proteasome function by blocking the channel, thereby inhibiting substrate access to the proteolytic active sites.

The NH_2 terminus of α3 forms interactions with NH_2 termini of other α-subunits. Of special note is a salt bridge formed between Asp-7 of α3 with Tyr-4 and Arg-6 of α4 (142, 143). Six of the seven α-subunits contain adjacent Tyr and Asp residues in homologous locations within their NH_2-terminal tails (all 7 contain this conserved tyrosine, and 3 also have an additional arginine immediately after the Asp residue); the conservation of this "YD(R)" sequence suggests a key structural role for this element. In addition to the bridge between the NH_2-terminal tails of α3 and α4, other pairs of α-subunits could also interact through their YD(R) motifs. The direct contacts formed between these residues in adjacent subunits may explain their correlated evolutionary conservation. Likewise, the NH_2 terminus of the single α-subunit in the archaeon *T. acidophilum* contains a Tyr8-Asp9-Arg10 (YDR) sequence, making it likely that the identical neighboring α-subunits in the archaeal CP also interact with each other via similar salt bridges. Thus in reality the α-subunit NH_2 termini are not randomly ordered but closely interact with each other to maintain a closed structure at the center of the α-ring in free CP.

3. 19S RP

The 19S RP serves multiple roles in regulating proteasomal activity: selecting substrates, preparing them for degradation, translocating them into the CP, as well as probably influencing the nature of products generated by the CP. The subunit composition of the RP from different species is remarkably similar (76, 91, 103, 125, 127, 135, 174, 455a). The RP is comprised of at least 18 different subunits with a total mass of close to 1 MDa and can assemble at either end of the 20S CP to form the 26S proteasome (76, 127, 342, 363). Purified proteasomes are always found as a mixture of free CP, singly capped (RP_1CP), and doubly capped (RP_2CP) forms (127), while in vivo it seems that in *S. cerevisiae* the majority of proteasomes are present as doubly capped forms (381). In mammalian cells, the ratio of RP to CP is lower probably leading the presence of free 20S CP and to proteasomes with a single 19S RP (47). In some cases, the surface of the opposing α-ring is then found bound to other activator complexes such as PA28 (Fig. 5) (160, 431). Six of the RP subunits are ATPases of the AAA family found in many multisubunit cellular machines such as translocaters, transporters, membrane fusion complexes, and proteases (33, 323a, 337) and are designated in yeast as Rpt1–6 (for regulatory particle triple-A protein). The other subunits of the RP are designated in yeast Rpn1–13 (regulatory particle non-ATPase). The six Rpt subunits together with three of the Rpn proteins (Rpn1, Rpn2, and Rpn10) form the base of the RP that attaches to the α-ring of the CP, while the remaining Rpn subunits can dissociate together as a lid subcomplex (see Fig. 5).

The overall conservation of the RP subunits in eukaryotes is extraordinary. The Rpt subunits are the most conserved subunits of the RP, each of which is 66–76% identical between yeast and humans, pointing to their central and enzymatic role in proteasome function. The non-ATPase subunits show a lower yet significant amount of sequence identity, typically in the range of 33–47%. A number of species-specific or loosely associated RP subunits have been identified in certain purified preparations; however, the basic 18 Rpt and Rpn subunits are present in all eukaryotic preparations. Some of the RP subunits show homology to each other (127). The six ATPases are roughly 40% identical to each other over the length of the protein, with the AAA domain at the center showing a greater degree of identity (377). These ATPases are distinct, as similar mutations in each result in unique phenotypes (127, 377). Among the non-ATPase subunits, three

pairs show close to 20% identity: Rpn1 with Rpn2, Rpn5 with Rpn7, and Rpn8 with Rpn11. The same relationship is maintained among their mammalian counterparts. This raises the idea that gene duplication plays a role in the composition of the contemporary proteasome.

The base is composed of two non-ATPase subunits (Rpn1 and -2) and six ATPase subunits (Rpt1–6) that are members of the diverse AAA-ATPase family. At least some of the Rpt subunits attach directly to the CP α-ring and function to open the central channel and translocate substrates into the CP (Fig. 5). It is presumed that the Rpt subunits assemble into a six-membered ring similar to the ATP-dependent ClpAP and HslVU proteases (224, 373). Known interactions between Rpt subunits indicate that such a hexameric ring should be orientated as Rpt1–2-6–4-5–3 in a clockwise manner in relation to the CP (72a, 109a, 136, 152, 369). Due to the symmetry mismatch (seven α vs. 6 Rpt subunits), it is not expected that each Rpt will form a stable interaction with a single a subunit. Reported CP-RP contacts include α1-Rpt6, α2-Rpt6, α4-Rpt2, α6-Rpt4, α2-Rpt4, α2-Rpt5, α4-Rpt4, and α7-Rpt4 (72a, 109a, 119, 152, 384, 499). That the NH_2-terminal tails of all seven α-subunits point directly into the center of sevenfold symmetry could explain how a single Rpt subunit can interact with more that one α-subunit.

Rpn10 is tightly associated with the base, although it can also bind to the lid, or be found separate from the proteasome (126, 150, 382, 428, 453). Rpn10 has a role in stabilizing the interactions between the lid and the base subcomplexes of the RP; the lid detaches to a greater extent from proteasomes purified from the *Δrpn10* strain (126). The first 190 amino acids of Rpn10 define a vWA domain, a structural motif termed after the von Willebrand factor (vWF), a multimeric protein that mediates platelet adhesion (178). Mutations within the NH_2-terminal region of the vWA domain of Rpn10 decrease the affinity of the lid for the base (110, 126). In these instances, the mutated Rpn10 copurifies with the base and not with the detached lid, suggesting a role for the vWA domain in stabilizing lid-base interactions. In a possible analogy to the vWA domain of Rpn10, the first vWA domain in vWF binds to the platelet glycoprotein Ib-IX-V complex. Mutations in this domain cause loss of binding of glycoprotein Ib to vWF, probably due to loss of structure in the vWA fold (96, 109a, 296a). The NH_2-terminal vWA motif of Rpn10 interacts with the lid and the COOH-terminal UIM region binds poly ub chains. The central part of Rpn10 links it to the base (109a).

While Rpn10 can interact with both the lid and the base of the RP, it most likely has an as yet unknown function outside of the proteasome as well, since it is the only proteasome subunit found in significant amounts unassociated with proteasome (150, 453). It has been shown that in vitro, proteasome-dependent degradation of polyubiquitinated substrates is inhibited upon addition of excess Mbp1, the *Arabidopsis* homolog of Rpn10 (86). This result has been attributed to free Rpn10 sequestering polyubiquitinated substrates from reaching the proteasome.

4. The lid of the proteasome

The lid consists of eight subunits arranged in a disklike shape that can detach from the RP base as a discreet complex and reattach to it (44, 126, 221). The eight proteins that make up the lid subcomplex contain one of two structural motifs: the PCI domain and the MPN domain (80, 126, 177). None of the RP base proteins contains these motifs, further differentiating the base and the lid as to their structure and possible evolutionary origin (13, 126, 177). Rpn10 aids in the correct assembly of the RP by stabilizing the link between the lid and the base via its NH_2 terminus (Fig. 5), although Rpn10 alone is neither essential nor sufficient for this task (109a, 126). The lid subunits are all non-ATPases, and their function or enzymatic activities are not yet known. Proteolysis of ubiquitinated proteins by the proteasome requires lid attachment, which suggests interaction with the multiubiquitin chain either through a ubiquitin-binding or deubiquitinating subunit (126). In comparison, certain nonubiquitinated proteins are efficiently proteolysed by eukaryotic proteasomes from which the lid has been detached (126), as well as by naturally "lidless" archaeal or prokaryotic proteasomes (78, 298). The simplest explanation is that a "lid" was added to the rudimentary RP concomitantly with the appearance of ubiquitin as a targeting system in eukaryotes. Alternatively, the lid could also play a structural role, such as defining a cavity within the RP, to allow proper binding of polyubiquitinated substrates.

The PCI and MPN structural motifs found in subunits of the lid subcomplex are also found in several other members of protein complexes, such as the eukaryotic regulator of translation eIF3, and the COP9 signalosome (CSN) (13, 126, 177, 225). The PCI domain is roughly 200 residues long and is predicted to form an α-helical structure found at the COOH termini of Rpn3, Rpn5, Rpn6, Rpn7, Rpn9, and Rpn12. The MPN domain is a 120-amino acid-long sequence that forms an α/β-structure at the NH_2 termini of Rpn8 and Rpn11. The PCI domain is found only in eukaryotic proteins, and it seems that they are all members of multiprotein complexes. Some regions within the MPN domain, on the other hand, are ancient and occur even in proteins from various bacteria, archaea, and phages. Most prominent is a family of proteins related to the tail assembly protein vtaK of bacteriophage λ. Some features of the MPN motif are also conserved in the bacterial DNA damage protein radC (Kay Hofmann, personal communication). Neither the functions of the MPN or PCI motifs nor the significance of the homology between the three MPN- and PCI-containing complexes have yet been elucidated. Within the lid, a tight structural cluster is formed around Rpn5, 8, 9, 11 synonymous with the similar cluster in the CSN, CSN4, 5, 7, 6 (109a). The identical pairwise interactions imply a conserved core structure and

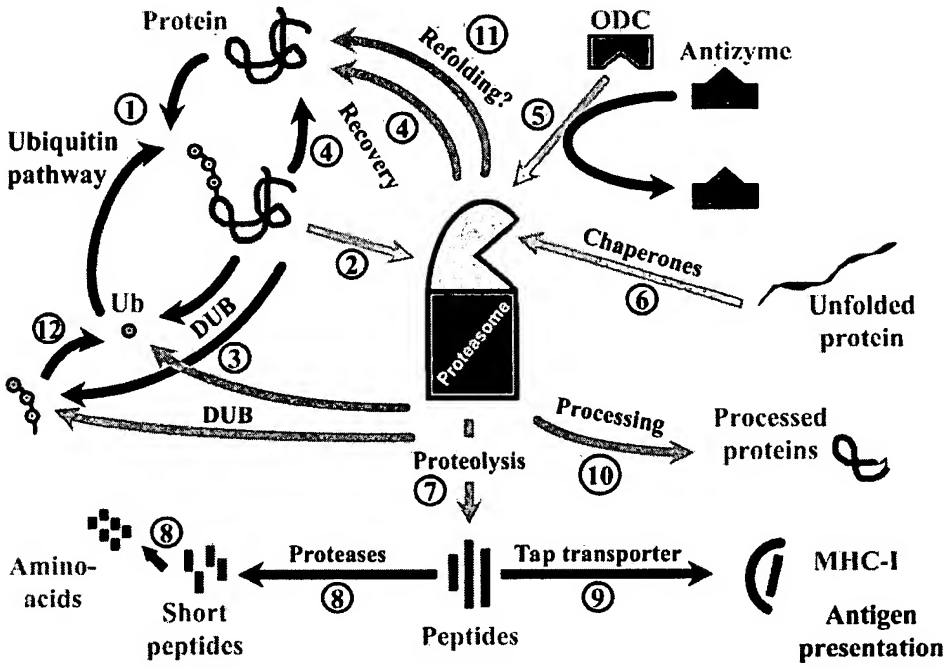

FIG. 6. Functions of the proteasome. The proteasome can carry out a broad range of tasks, both in substrate selection (green arrows) as well as in product generation (orange arrows). *1*: Proteins (red) are covalently conjugated to a multiubiquitin chain (ubiquitin; blue) by the ubiquitin-pathway enzymes. *2*: Ubiquitin-protein conjugates are specifically targeted to the proteasome. *3*: Ubiquitination is reversible; deubiquitinating enzymes (DUBs), whether in the cytoplasm (*3*; black) or proteasomal (*3*; orange), can remove ubiquitin from the substrate. Some DUBs probably release single ubiquitin moieties, whereas others release ubiquitin chains. *4*: Deubiquitinated substrates are recovered (black) or released (orange) from the proteasome. *5*: In some instances, substrates can be targeted to the proteasome in a ubiquitin-independent manner. ODC is targeted by antizyme, while for $p21^{Cip1}$, no ancillary protein has been reported. *6*: Unfolded proteins might be recognized directly by the RP, or after association with molecular chaperones. *7*: Proteolysis of proteins to short peptides of varying lengths. *8*: Further degradation of peptides to amino acids. *9*: Binding of peptides to the ER Tap transporter for presentation on MHC class I molecules. Peptides may be trimmed to obtain optimal binding to the MHC complex by cytosolic and/or ER protease(s). *10*: Limited processing of substrates (p105 precursor of NF-κB). *11*: Refolding of denatured proteins and their rescue from proteolysis. *12*: DUB-mediated processing of polyubiquitin chains, including head-to-tail biosynthetic ubiquitin precursors.

support ancestral relationship. Mutational analysis identified the PCI and MPA motifs of these subunits as critical for lid assembly (109a).

B. Mechanism of Action: Degradation of Ubiquitinated Substrates

1. Ubiquitin recognition

A polyubiquitin chain is synthesized on the majority of substrates recognized by the proteasome (Fig. 6). Monoubiquitination may serve as a cellular targeting or localization signal, but it does not seem to target proteins to the proteasome (see sects. v and vii) (166, 349, 426). Chains containing at least four molecules of G76-K48 isopeptide-linked ubiquitin are necessary for efficient binding to the proteasome (435) or its component S5a/Rpn10 (19). The relationship between binding to the proteasome and chain length is not additive, since chains of four ubiquitins bind 100 times better than chains of two ubiquitins, whereas there is only a 10-fold increase when eight more Ubs are added to the chain (349, 435). Evidently, it is a surface provided by the four-subunit structure of a polyubiquitin chain that is recognized by the proteasome, rather than a single ubiquitin molecule. Evidence points to hydrophobic interactions that target the polyubiquitin signal and the receptor site in the proteasome. For instance, a hydrophobic cluster formed by a number of residues in the vicinity of Ile-44 of ubiquitin is necessary for efficient proteasome-dependent degradation (415). Likewise, a hydrophobic patch at the COOH-terminal region of Rpn10 and its orthologs (within its UIM motif) binds to ubiquitin chains (110, 492).

The quaternary structure of ubiquitin polymers and the exact spatial relationship between each ubiquitin molecule is also critical for their ability to target substrates for degradation by the proteasome. Modification of proteins by polyubiquitin chains linked via Lys-63 instead of Lys-48 plays a role in signaling, DNA repair, and maybe additional tasks but probably does not target proteins for degradation by the proteasome (see sects. v and vii) (79, 179, 417, 418). It is important to note, however, that multiubiquitin chains linked via Lys-6, Lys-11, or Lys-48, all bind to the proteasomal subunit Rpn10/S5a with similar affinities (19). Furthermore, linear ubiquitin fusions are competitive inhibitors of Lys-48-linked multiubiquiti-

nated substrates for proteasomal binding, indicating they probably bind to the same site on the proteasome (435). These observations indicate that there is a separation between binding to the proteasome and correctly preparing a substrate for degradation (Fig. 6).

Recognition of a protein by the proteasome does not guarantee its efficient degradation. For instance, there are examples of both nonubiquitinated proteins as well as a pentaubiquitinated protein that are efficiently recognized by purified proteasomes in vitro, but are not efficiently proteolysed (44, 425, 435). Even in vivo the transcription factor Met-4 is polyubiquitinated presumably with a G76-K48 chain (by SCF^{Met-30}), but nevertheless is not rapidly degraded by the proteasome (214).

To date, the only known subunit in the proteasome with affinity for ubiquitin chains is Rpn10/S5a/Mcb1 (84, 85, 150, 452, 453, 473). Rpn10 contains a ubiquitin binding site found within its COOH terminus in a hydrophobic patch containing a LALAL (or similar) motif (110). This ubiquitin-binding motif is part of a larger ubiquitin-interacting motif (UIM) common to a number of proteins in the ubiquitin or lysosomal pathways for degradation (178). The COOH termini of Rpn10 homologs, such as p54 from *Drosophila* and S5a from mammals, contain two such hydrophobic repeats within their UIM, both of which have been shown to bind ubiquitin (150, 492). Even though this ubiquitin binding site is stringently conserved in the COOH termini of Rpn10 homologs from a number of species (110, 150, 492), deletion of the UIM of Rpn10 has no discernible phenotype in *S. cerevisiae*. Deleting only the NH_2 terminus of Rpn10, on the other hand, causes a phenotype similar to deletion of the entire *RPN10* ORF (110). This indicates that Rpn10 functions in the proteasome independently of its ability to bind ubiquitin chains. In addition, as *RPN10* or its orthologs are nonessential in *S. cerevisiae*, *S. pombe*, and moss, it was suggested that it does not function as the sole ubiquitin recognition site in the proteasome (123, 453, 473). More than one proteasomal subunit might be necessary for recognition and binding of ubiquitin chains. Indeed, Rpn10 in combination with a comprised version of Rpn1 is essential, although it is unclear whether they share functions or merely interact with one another in the base (473).

Rpn10 may play overlapping roles with an unrelated protein, Rad23 (257). Although Rpn10 is the only proteasomal subunit known to have affinity for multiubiquitin chains, Rad23 can be coprecipitated with substrates that contain short multiubiquitin chains (329) and a ubiquitin-associated domain (UBA) at the COOH-terminal part of the protein can directly interact with ubiquitin (32, 56a, 176, 471a). Rad23 (as well as its human counterparts hHR23a and hHR23b) can also bind tightly to yeast or mammalian proteasomes through an NH_2-terminal UBL (ubiquitin like) domain that shares homology with ubiquitin (172, 386). Mammalian hHR23 can bind Rpn10/S5a through this UBL domain, but it can also bind to the proteasome independently of Rpn10 (257), indicating that there might be an additional site for binding of ubiquitin or ubiquitin-like proteins in the 19S RP.

It is not at all clear what is the role of Rad23 binding to the proteasome or to Rpn10, although it potentially links the proteasome to the DNA repair pathway by targeting the proteasome to sites of DNA damage. Whether the ability of Rad23 to interact with lightly ubiquitinated substrates has anything to do with this function is yet to be shown (329, 380, 386). It should be reemphasized that there is no evidence that monoubiquitin targets proteins to the proteasome. Therefore, binding of molecules with a single noncleavable ubiquitin-like (UBL) domain, such as Rad23, could be distinct from that of multiubiquitin chains. Alternatively, binding of molecules with noncleavable UBL domains could simply be enhanced since this domain is not removed, whereas the 19S RP-associated DUB can rapidly deubiquitinate monoubiquitinated substrates.

A yeast two-hybrid screen has indicated that Rad23 can interact with Rpn1, a subunit of the base (51a). In addition, Rpn1 from *C. elegans* can interact with linear polyubiquitin while Rpn2 can interact with another UBL-containing protein (72a). Thus Rpn1 and Rpn2 might be additional sites for polyubiquitin or UBL binding in the proteasome. hPLIC, which also contains a UBL domain, has been found to interact with the proteasome as well (236). Similarly to Rad23, hPLIC and its yeast homolog Dsk2 contain in addition to their NH_2-terminal UBL domain, a COOH-terminal UBA domain as well. Interestingly, both Rad23 and Dsk2 can interact with polyubiquitin chains via their UBA domains (471a). Possibly, they serve as a parallel pathway for targeting polyubiquitinated substrates to the RP, although this is still unproven. Pleiotropic defects have been observed for deletions of *rpn10* and *rad23* in *S. cerevisiae* or for deletions of *rpn10*, *rad23*, and *dsk2* in *S. pombe* (257, 471a).

Similarly to the base, another hexameric ring of AAA ATPases VCP (also known as p97 in animals, Cdc48 in yeast, or VAT in archaea) has been shown to contain an unfoldase ability (130a, 503). VCP has been shown to physically bind both to the proteasome, as well as to polyubiquitinated substrates and polyubiquitinated chains directly (69a). It is not impossible, therefore, that members of the putative ring of Rpt ATPases in the base also have a certain affinity for polyubiquitin chains. Together this suggests that binding of polyubiquitin and ubiquitin-like domains occurs in the base, with at least some of this activity taken up by Rpn1, Rpn2, and Rpn10. Whether they each have unique affinities for different UBL domains or alternatively assembled polyubiquitin chains is unclear at this time, nor whether binding of all polyubiquitin chains and single UBL-domains occurs at the same subunits of the 19SRP.

2. Substrate binding

Rpn1 and Rpn2 make up the RP base together with the six Rpt ATPases. Both Rpn1 and Rpn2 contain multiple leucine-rich repeats (LRR), a domain suggested for protein-protein interaction (283). Similarly to the regulatory domain of simple ATP-dependent proteases in prokaryotes, which directly interact with substrates (137), the six ATPases of the base are also likely to function through protein-protein interaction with the substrates of the proteasome. Thus it is plausible that all eight components of the base may engage in direct interactions with substrates. Distinct functions of the base and the lid must be consistent with their location within the RP. The substrate must be properly positioned to be unfolded by the base and translocated into the CP; the distal positioning of the lid may ensure that the ubiquitin chain on the substrate does not occlude access of the target protein to the channel. Indeed, the base can bind to nonubiquitinated, unfolded substrates and promote their folding (44, 425). PAN, an archaeal homolog of the base, can directly interact with folded proteins and unfold them (318a). Whether interacting with substrates via their ub moieties or in a ubiquitin-independent manner reflects distinct mechanisms for preparing them for degradation remains to be seen.

3. Unfolding and translocation

Not only is ATP hydrolysis also required for assembly of the proteasome from isolated RP and CP (15, 175), proteolysis of proteins by the proteasome is also strictly ATP dependent (76, 127, 164). In analogy to molecular motors and chaperones (6, 11, 434), it is likely that ATP hydrolysis cycles the RP between high- and low-affinity states, alternately binding and releasing substrate. The conformational changes associated with this ATPase cycle could be used in three processes: *1*) gating the channel defined by the NH_2 termini of the core particle's α-ring subunits, *2*) unfolding the substrate, and *3*) threading the unfolded substrate through the channel into the lumen of the CP. It would be interesting to know whether these functions of the ATPases are coupled, namely, whether upon binding of substrate the ATPases unfold it and gate the channel simultaneously, or whether separate ATPases play distinct roles. Because archaeal proteasomes and other ATP-dependent proteases do not contain a lid-like subcomplex, it seems that the above-proposed functions do not require the lid. Indeed, clpA, VAT, and PAN have both been shown to unfold folded nonubiquinated proteins (318a, 466).

Unfolding of protein substrates is required, since the estimated inner diameter of the CP channel is too narrow for proteins to enter in their native state. ATP binding and hydrolysis by the ATPases may trigger cycles of higher and lower affinities of the RP for substrate proteins, thus stabilizing them in an unfolded state. A possible role for the base in promoting substrate unfolding is suggested by its location, covering the entry ports into the CP, and by the presence of six ATPases within this complex. A simple homolog of the base in archaea, PAN, has been shown to unfold proteins prior to translocating them into the CP (318a, 504). In vitro, both the RP and the base can bind unfolded proteins and release them in an ATP-dependent fashion (44, 425). The refolding activity of the base may be an independent function of the RP or it may reflect a normal function of the base as part of the unfolding process of proteolytic substrates. For instance, the directionality of the reaction, whether the base serves to fold or unfold, may be dependent on the structure of the substrate as it binds the RP (i.e., native or denatured). The directionality could also be influenced by the end products: proteolysis is irreversible, driving the reaction toward unfolding and translocation into the CP. Substrates are thus translocated from one domain within the proteasome (RP) to another (CP) probably in an ATP-dependent manner.

4. Gating

An important property of the base is that it is nearly as efficient as the intact RP in stimulating the degradation of peptides and a nonubiquitinated protein substrate by the CP, suggesting a role for proteasomal ATPases in preparing substrates for degradation (126). One role of the RP might be to control opening and closing of this channel by forming competing interactions with the blocking α-subunit NH_2-terminal tails (142). Indeed, a substitution mutation in the ATP-binding site of a single ATPase (*RPT2*) severely lowers peptidase activity of the proteasome, probably due to hampering the ability of the RP to properly gate the channel into the CP (242, 377). This indicates that even the entry of small peptides, which do not need to be unfolded, can be controlled by the RP.

Furthermore, an "open gate" mutation generated by deletion of the nine NH_2-terminal residues of the α3-subunit (α3ΔN) has only a small effect on peptidase activity of proteasome holoenzymes (142). The specific activity of purified α3ΔN CP is similar to that of 26S proteasome purified from the same mutant strain, as well as being similar to the specific activity of wild-type 26S proteasome holoenzyme. It would seem that attachment of the RP activates peptidase activity of the CP to a similar extent as caused by the α3ΔN open gate mutation. One possible mechanism for this activation is that attachment of the RP to the surface of the α-ring forms competing interactions with the Tyr, Asp, or Arg residues situated in the α-subunits (see sect. VI*A*), thus realigning their NH_2 termini to allow passage of substrates or products. So far, the pair Rpt2-α3 has been shown to be involved in gating the channel into the CP, although it is possible that gating is controlled by multiple Rpt-α interactions (72a, 109a, 142, 242, 377).

One reason for a gated channel in the CP could be to

serve as a transition from one form of inhibition to another during assembly of the mature CP. In the final stage of CP assembly, self-compartmentalization is achieved by the association of two $\alpha_7\beta_7$ half-CPs at the β-β interface. These half CPs are inactive due to propeptides in the critical β-subunits that mask their active site. As these half-CPs are joined, inhibition by β-subunit NH_2 termini is relieved by autolysis (57, 143) while inhibition by the blocking NH_2 termini of the α-subunits is imposed. Binding of the RP relieves this inhibition by opening the channel, thus giving rise to the proteolytically active form of the complex (142, 242).

A second reason for a gated channel could be to regulate generation of products by the proteasome. It is possible that under normal conditions product release is slowed down by a gated channel to increase processivity and decrease average peptide length. Most of these short peptides are quickly removed from the cytoplasm (see Fig. 6). Under certain conditions (such as during immune response), it might be beneficial to produce longer peptides that can play a regulatory role. The majority of peptides generated by the proteasome contain less than eight amino acids. A fraction of the peptides that are 8–10 amino acids in length can be transported through the ER and presented to the immune system by MHC class I (124, 364). An increase in average peptide length of proteasome products could increase the efficiency of antigen presentation and, by extension, the efficiency of combating viral infection. Upon interferon-γ induction, after viral infection for example, a regulator of the proteasome, PA28 (also known as the 11S regulator), plays a role in antigen processing by activating the peptidase activity of the 20S CP (141, 364, 423). PA28 probably activates the CP by binding to the α-ring surface and rearranging the blocking NH_2 termini (469). It is possible that opening the gate increases the rate by which peptides exit the CP and alters their make up to better fit antigen presentation requirements (242).

5. *Proteolysis*

In most cases, proteasomes cleave protein substrates into small peptides varying between 3 and 23 amino acids in length (231, 322). The median length of peptides generated by the proteasome are seven to nine amino acids long; however, in total peptides within this size range make up ~15% of the peptides generated by the proteasome. This process is processive such that a protein is hydrolyzed within the proteasome to the final products before the next substrate enters; thus the pattern of peptides generated from a specific protein is stable over time (230, 322). The peptide products of the proteasome are short lived and do not accumulate in the cell (412, 429). Most likely, most of these peptides are rapidly hydrolyzed by downstream proteases and aminopeptidases (Fig. 6, arrow 8). Candidates for these downstream proteases are THIMET, Tricorn, Multicorn, TPPII, and leucine aminopeptidase (26, 331, 412, 429, 484). Some of the peptides that are generated by the proteasome can be transported through the ER to be presented to the immune system by the MHC class I molecules (306, 424).

Overall, the 20S CP can cleave peptide bonds after any amino acid. However, each of the three active site-containing β-subunits preferentially cleaves after different amino acids: β1 cleaves after acidic or small hydrophobic amino acids, β2 cuts after basic or small hydrophobic amino acids, while β5 hydrolyzes the peptide bond after hydrophobic residues whether bulky or not (88). The rules that govern the cleavage rate of the same peptide bond can be significantly altered when put into the context of the primary structure of the polypeptide (180). For instance, the specificity toward a peptide bond between two amino acids can be affected by the amino acids in flanking regions on either side up to eight amino acids away. The location and identity of each of these anchoring residues are different for different classes of peptide bonds (180, 247). An interesting feature of proteolysis by the proteasome is that the 20S CP and the proteasome holoenzyme generate different patterns of cleavage products (95, 231), indicating that even the distal 19S RP affects the behavior of the CP. Furthermore, the CP contains specific "noncatalytic" sites to which additional factors can bind and alter cleavage sites and product composition (392). Understanding the precise rules regulating the makeup of peptides generated by the proteasome will have far-reaching consequences on predicting immunogenic peptides "hidden" within viral or tumorigenic proteins.

6. *Proteolysis and antigen presentation*

A significant portion of antigenic peptides to be presented by MHC class I molecules is generated by the proteasome (238, 364, 489). The proteasome removes foreign proteins, such as those of viral origin, from the cell by hydrolyzing them into short peptides. Only a fraction of the peptides generated by the proteasome are of the correct length to be transported through the ER and presented to the immune system by MHC class I. The regulatory role of PA28 in antigen processing might be to affect the nature of products generated by the proteasome by altering the peptidase activity of the 20S CP to increase the efficiency of antigen presentation and by extension combating viral infection (141). Indeed, hybrid proteasomes with one 19S RP and one PA28 at opposite ends of the CP have been identified (160, 431), suggesting that an intricate process of selecting substrates by the RP and regulating products by the PA28 is necessary for efficient antigenic peptide production.

Due to amino acid differences in the S1 pocket (which defines substrate specificity) of the three βi-subunits compared with their constitutive β-counterparts, a drastic decrease in postacidic, and to some extent postbasic, endopeptidase activity is measured for immunoproteasomes; immunoproteasomes cleave peptides mainly after hydro-

phobic positions (115). Because peptides are anchored in the MHC I groove preferentially by hydrophobic terminal residues, immunoproteasomes could be instrumental in the generation of antigens for MHC I presentation (306, 424). Indeed, the 26S proteasome can specifically hydrolyze a protein to yield a known MHC class I antigenic peptide (28), and inhibition of the proteasome blocks presentation of antigenic epitopes (53). However, it is not necessary that the proteasome directly produce antigenic peptides, as some of its longer products could be further processed to yield the correct epitopes that are presented to the immune system. For instance, the small percentage of peptides that are longer than eight or nine amino acids and containing immunogenic epitopes could be cleaved further to generate antigenic peptides (340). It is even possible that the TAP transporter actually prefers to transport peptides that are longer than the optimal T-cell epitope, and these peptides must be further trimmed or refined in the ER to yield the eight or nine amino acid long MHC class I-type peptides (340, 464). Nevertheless, evidence to the contrary exists indicating that many MHC ligands are generated in their final form directly by the proteasome (281). According to this work, not only is the proteasome responsible for both the NH_2- and COOH-terminal cleavage sites, but also these peptides are efficiently transported by TAP and bind to MHC molecules. In any event, generation of antigenic peptides involves a hierarchy of selection steps: ubiquitination, proteasome degradation, TAP transport, optional ER trimming, and MHC I binding efficiency. Due to the competition for the peptide products of the proteasome, transport into the ER must be faster than the rate at which they are hydrolyzed by cellular proteases or amino peptidases (Fig. 6).

7. Deubiquitination

Finally, ubiquitin is also a product of the proteasome; ubiquitin, or ubiquitin attached to a residual peptide chain, is released from the proteasome and recycled back into the ubiquitin pathway (185, 427). A deubiquitinating enzyme must be associated with the RP to remove or edit these polyubiquitin chains (Fig. 6). The RP from a number of sources has been shown to contain a ubiquitin hydrolase activity that can serve to edit these ubiquitin chains, or remove ubiquitin from protein substrates (98, 174, 254, 256, 259, 272). In *Drosophila*, the RP subunit responsible for this activity was identified as p37a; however, an obvious ortholog of this protein is not present in *S. cerevisiae*. Another deubiquitinating enzyme, USPI4, has been shown to interact with mammalian proteasomes (39a). Alternatively, Doa4 has been suggested as the deubiquitinating subunit of the RP in *S. cerevisiae* (335). The exact mechanistic role of this deubiquitinating activity in proteasome function is still not clear. It is possible that the polyubiquitin chain must be removed from the substrate by the RP before the substrate can be translocated via the narrow channel into the proteolytic chamber of the CP. However, it is also possible that removal of the polyubiquitin tree by deubiquitination actually releases substrates that have been improperly ubiquitinated (Fig. 6).

C. Additional Functions of the Proteasome

1. Degradation of nonubiquitinated proteins

The most important role of the proteasome in eukaryotes is to proteolyse proteins that are covalently attached to a chain of multiple ubiquitin moieties. Simple versions of the proteasome are present in archaea and certain bacteria, while ubiquitin and the ubiquitin conjugating system are not, indicating that the proteasome has the ability to proteolyse at least some nonubiquitinated proteins (298, 503). In these organisms, the proteasome is involved in the heat shock response by degrading damaged or misfolded proteins (240, 298, 379). In vitro, purified core particles from both prokaryotes and eukaryotes can hydrolyze a number of nonubiquitinated unfolded proteins in vitro (87, 230, 231). PAN, an archaeal regulatory complex, can promote nonubiquitinated protein unfolding and degradation by the proteasome (318a, 504). In eukaryotic cells, however, all indications are that the bulk of short-lived proteins, including damaged and misfolded proteins, as well as mistranslated newly synthesized polypeptides, are removed from the cell in a ubiquitin-dependent manner (62, 393, 403, 490).

At least one example of a metabolic protein that is targeted to the proteasome in a specific, yet ubiquitin-independent manner, exists; the enzyme ornithine decarboxylase (ODC) is recognized and hydrolyzed by the proteasome upon binding to the protein antizyme, without ubiquitination (Fig. 6, arrow 5; Ref. 315). Antizyme itself is probably released from the proteasome and not degraded concomitantly with ODC (315); however, it is possible that the levels of antizyme could be independently regulated via proteasome degradation. As the machinery for ubiquitin-independent targeting to the proteasome is present in eukaryotes, one can assume that the case of ODC will not be a unique exception and that antizyme, or other small molecules, could target a subset of substrates to the proteasome. Another example is the degradation of the Cdk inhibitor $p21^{Cip1}$. p21 is a short-lived unstable protein that is normally rapidly ubiquitinated and degraded by the proteasome. Nonetheless, mutants of p21 have been generated that are not ubiquitinated but are efficiently proteolysed by the proteasome (406). It is possible that in addition to ubiquitination, direct targeting of $p21^{Cip1}$ to the proteasome takes place (439).

2. Processing

An interesting feature of the proteasome is that not all substrates are hydrolyzed to completion (Fig. 6). In

some cases, the proteasome processes the substrate into a truncated form. Processing by the proteasome can serve as a potent regulatory tool for transforming a protein from one form into another, thus altering its cellular activities. The best-studied example is the processing of the p105 precursor of p50, a component of the transcription factor NF-κB (334). After p105 is ubiquitinated, probably within its COOH-terminal half, this half is proteolysed by the proteasome, and the 50-kDa NH_2-terminal region is released as a stable and active subunit of the NF-κB transcription factor protein. In the case of p105, the site of processing is determined in part by a glycine-rich region (GRR) in the middle of the protein (275, 328), as well as by specific interactions of certain amino acids within the p50 domain that stabilize its three-dimensional structure, so it cannot be unfolded (261). Similarly, the p100 precursor of the p52 component of NF-κB2 is processed by the proteasome after a GRR (165). Interestingly, the yeast proteasome processes p105 in the absence of a GRR and probably at a different location than the mammalian proteasome does, suggesting that the GRR is probably not a universal signal for processing (399).

A unique case of processing a membrane-bound protein by the proteasome is the activation of Spt23, a transcription factor that controls the levels of unsaturated fatty acids. After ubiquitination and proteolysis by the proteasome, the active p90 NH_2-terminal region of Spt23 is liberated from the inactive membrane-bound intact p120 form (181). The assumption is that a loop in the full-length precursor is endoproteolytically clipped by the proteasome, releasing the NH_2-terminal active region and degrading the membrane-bound COOH terminus. Despite the structural similarity between NF-κB and Spt23, the latter does not contain a GRR but does have an asparagine-enriched region that might be central to processing of the loop (181). In yet another example, the 155-kDa protein Cubitus interuptus (Ci) is a transcriptional activator in the Hedgehog signaling pathway. Genetic evidence indicates that upon ubiquitination, its COOH terminus is proteolysed by the proteasome, while the 75-kDa NH_2-terminal domain is released as a transcriptional repressor in the Hedgehog pathway (291, 463). A fifth example of processing by the proteasome is the production of a specific 90-kDa truncated fragment from the larger ubiquitin-β-galactosidase fusion (20, 453). Although in this case the substrate is a model protein and its processing has no physiological relevance, this example further highlights the ability of the proteasome to process, in a limited manner, a small subset of substrates and not to destroy them completely. Thus the proteasome not only "destroys" proteins, but can also activate proteins or drastically alter their cellular behavior.

The mechanisms of processing are far from being understood. For instance, it is not clear whether processing is a distinct event that occurs before proteolysis, or whether it is simply a termination of the processive proteolysis of a substrate. In the former scenario, as suggested for Spt23, a substrate loop is pulled into the 20S CP, cleaved, and the nonubiquitinated half of the substrate is released from the 19S RP while the ubiquitinated half would remain to be degraded. In the second case, possibly the processing of p105, proteolysis by the 20S CP progresses processively until it reaches a "termination signal," whereby the remainder of the protein is released from the proteasome. It is not even clear whether processing takes place within the 20S CP, or at the level of the 19S RP, by an auxiliary protease.

Although certain internal sequences (such as the GRR) or residues that stabilize the three-dimensional structure appear to be "markers" for processing and demarcate a region within a protein that should be spared from degradation, other, similar, sequences could inhibit proteasome-dependent degradation altogether. The Epstein-Barr virus nuclear antigen 1 (EBNA1) is a stable viral protein that evades the ubiquitin/proteasome pathway for degradation and thus is not presented to the immune system. The stability of this protein has been attributed to a Gly-Ala repeat (GAr) that prevents its degradation by the proteasome (268). Insertion of synthetic GAr of varying lengths, from 8 to 239 amino acid extensions, caused a number of different ubiquitin/proteasome substrates, IκBα, for example, to be stabilized (71). Location of the GAr did not appear to be critical in those substrates in which it was inserted. This is in contrast to the similarly structured GRR in p105 that confers processivity. Transfer of the GRR within p105 abolished its effect, suggesting that its location within the molecule, proximal to an additional signal, is important for the processing reaction (328). The protection conferred by GAr is not absolute; certain signals for degradation (such as N-end rule destabilizing residues) can override even long GAr regions (71).

Another repeat signal that could serve to protect proteins from degradation is polyglutamine. The protein Ataxin-1 is normally degraded by the ubiquitin/proteasome system. Mutant Ataxin-1 containing a 90-polyglutamine repeat causes it to accumulate and aggregate in transfected cells, despite the fact that mutant Ataxin-1 can be polyubiquitinated. Nevertheless, degradation of the polyubiquitinated and poly-Q-containing molecule is significantly slower compared with its WT counterpart (68). Similarly, the mutant androgen receptor (AR) that contains 48 glutamines (ARQ48) accumulates in a hormone-dependent manner in both cytoplasmic and nuclear aggregates, probably due to a perturbance in proteolytic processing (421). Thus, although ubiquitination is necessary, it is not always sufficient to target a protein for degradation. Of note is that many of the above examples contain repeat sequences, whether polyglycine, polyasparagine, or polyglutamine. Quite possibly, the proteasome has difficulties processing or proteolysing extended repeat sequences due to a "glitch" in the recognition mechanism. Thus overall stability of a substrate can be a

delicate balance between the protecting and destabilizing signals.

3. Refolding/recovery

It is possible that the proteasome might also function in processes that do not involve proteolysis at all (Fig. 6). At least in vitro, the proteasome can bind certain unfolded proteins, accelerate their refolding, and release them in their native form (44, 425). The proteasome can also inhibit aggregation of misfolded proteins. These chaperone-like activities have been mapped to the ATPase-containing base of the RP (44). There is evidence that in vivo, the proteasome is involved in disassembly and rearrangement of the nuclear excision repair complex, without performing proteolysis (380). Similarly, RP subunits, but not CP subunits, colocalize in vivo together with heat shock proteins and chaperones at sites of misfolded AR aggregates (421). Components of the RP function in nucleotide excision repair in yeast independent of proteolysis (122). These may be in vivo examples of the in vitro observed chaperone-like activity of the RP. As the ATPase-containing regulatory complexes ClpA and ClpX of the prokaryotic ATP-dependent protease ClpP have dual functions, serving either as aids in proteolysis or as chaperones (137, 138, 466), it is possible that a similar distribution of functions occurs for the ATPase-containing RP pointing to nonproteolytic functions of the proteasome.

D. Interactions With Other Cellular Factors

Despite the fact that the composition of proteasomes purified from different species is almost identical, and the basic components of the proteasome are remarkably conserved among all eukaryotes, numerous proteins can bind to the proteasome with different affinities and ratios (72a, 102, 455a). There is increasing evidence that the proteasome is in fact a dynamic structure forming multiple interactions with transiently associated subunits and cellular factors necessary for functions such as cellular localization, presentation of substrates, substrate-specific interactions, or generation of various products (Fig. 5).

1. Components of the ubiquitin system

The proteasome may interact with the enzymatic complexes that present substrates to it for degradation. For instance, it is still unclear how ubiquitinated proteins are brought to the proteasome. There are now increasing reports that there are direct contacts between the ubiquitin system and the proteasome, suggesting that ubiquitinating and degradation might not be completely independent processes. E2s such as Ubc1, Ubc2, and Ubc4 coimmunoprecipitate with the proteasome (438). The interaction between Ubc4 and the proteasome seems to be enhanced under stress conditions, when the need to remove damaged proteins increases (438). In addition, interactions of two E3s, Ubr1 and Ufd4, mapped to components of the base of the RP, specifically to Rpt6, Rpt1, and Rpn2 (479). A mammalian HECT domain E3, KIAA10, can interact with the proteasome, specifically with s2/Rpn1, via its NH_2 terminal (491). Proteomic screens have identified subunits of the APC and SCF E3 complexes as copurifying with the 19S RP and the *C. elegans* homolog of the putative E3 ubiquitin ligase as an interactor with the lid subunit Rpn11 (72a, 455a). The SCF complex is also expected to interact indirectly with the proteasome via a newly discovered recruiting factor, Cic1, which is essential for the degradation of the Grr1 and Cdc4 F-box components of the SCF by interacting with both complexes (199).

Indirect interactions between the ubiquitination machinery and the proteasome are also implied from studying the behavior of hPLICs. HPLIC-1 and hPLIC-2, two human homologs of yeast Dsk2, have been shown to interact both with the proteasome as well as with two different E3s (E6-AP and β-TrCP), and they can all colocalize as part of a multiprotein complex in vivo (236). It is interesting to note that similarly to other proteasome interactors (such as Rad23 and Bag1; see below), Dsk2/hPLIC also contains a noncleavable ubiquitin-like domain at its NH_2 terminal. Because substrate specificity of the ubiquitin-proteasome pathway resides mostly at the level of the E3 enzymes (Figs. 2 and 3), targeting of E3s to the proteasome directly or via recruiting proteins may be a way to deliver substrate specificity to the proteasome.

Rad23 can bind tightly to both purified and partially purified proteasomes, or to one of its subunits, s5a/Rpn10 (172, 380, 386). The function of this interaction is not clear, although it is implicated in linking the proteasome to the DNA-repair pathway and the nuclear excision repair complex (380). The UBA (ubiquitin-associated) domain at the COOH terminus of Rad23 has been shown to bind ubiquitin or polyubiquitin chains (32, 56a, 471a). Because Rad23 also includes a UBL domain at its NH_2 terminus, through which it possibly binds to the proteasome (172, 257, 380, 386), Rad23 might be involved in targeting ubiquitinated proteins to the proteasome. An additional protein that has UBL domain and can bind to the proteasome is the chaperone cofactor BAG-1 (282). Both BAG-1 and Rad23 contain noncleavable UBL domains at their NH_2 termini that are required for their attachment to the RP. BAG-1, in addition to promoting the association of Hsc70/Hsp70 with the proteasome, also interacts with CHIP, a U box-containing ubiquitinating enzyme (66, 153, 303). These interactions of BAG-1 point to the linkage between proteasome, chaperone, and ubiquitination functions.

A fascinating story has recently unfolded by studying the interaction of Bag-1 with the proteasome. It has long been an enigma which class of E3s recognizes and ubiquitinates unfolded or damaged proteins. Chaperones such as Hsp70 and Hsp90 recognize misfolded proteins and

refold them in an ATP-dependent manner, often aided by cochaperones (299a). One such cochaperone is Bag-1, a UBL-containing protein that can simultaneously interact both with chaperones such as Hsp70 and the proteasome (282). In parallel, U-box proteins have recently emerged as a group of E3 ligases, related to the Ring finger enzymes but without specific substrate-recognition ability (153, 204a). It was later shown that a member of this family, CHIP, ubiquitinates chaperone-bound unfolded proteins (75b, 315a). CHIP can cooperate for this purpose with Hsp70 or Hsp90. Thus the Hsp70-CHIP pair represents an E3 ligase for recognizing unfolded proteins. This E3 complex probably targets its cargo to the proteasome by means of Bag-1. Two additional UBL proteins interact with Hsp70 as well, Chap1/Dsk2 (hPLIC) and Chap2/Scythe (223b). Because hPLIC is known to target other E3s to the proteasome, it might be possible that this novel E3 complex formed by joining Hsp70 and CHIP is targeted to the proteasome in more than one manner.

The proteasome also interacts with the other end of the ubiquitin system, the deubiquitinating enzymes (DUBs; Fig. 6). A number of reports indicate that the proteasome from a number of different species, and more specifically the RP, have deubiquitinating (DUB) activity, i.e., the ability to hydrolyze peptide or isopeptide bonds after the COOH terminus of ubiquitin (98, 174, 254, 256, 259, 272). The subunit responsible for this activity in *Drosophila* has been identified as p37a (174). p37a, UCH37, or a homolog might be the subunit responsible for the polyubiquitin chain editing function associated with proteasomes purified from different sources (174, 254, 272). A clear ortholog of p37a has not been identified in *S. cerevisiae*. Furthermore, the proteasome exhibits a wide range of DUB activity, including typical UBP-class deubiquitinating activity, whereas p37/UCH37 is a member of the UCH family (259). It is possible that more than one DUB is associated with the proteasome. Indeed, in addition to UCH37, also USP14 has been reported to interact with the 19SRP (39a). A homolog of USP14, ubp6, is present in the *S. cerevisiae* genome, but so far there are no reports of its binding to the 19SRP. In *S. cerevisiae*, Doa4, a deubiquitinating enzyme of the UBP family, has been proposed to function in conjunction with the proteasome by trimming multiubiquitin chains from proteasome-bound substrates (335, 336). Doa4 interacts weakly and in substoichiometric amounts with the proteasome (335). Doa4 might serve to release ubiquitin and regenerate the proteasome for the next catalytic cycle (335).

2. Chaperones and heat shock proteins

Despite the fact that the RP of the proteasome might have intrinsic chaperone-like activity, increasing evidence links chaperones with proper proteasome function. A number of studies report copurification of Hsp90 with the proteasome (311, 459). In yeast, overexpression of wild-type but not mutant Hsp70 and DnaJ can suppress growth defects associated with mutant CP subunits (324). One model proposes that molecular chaperones shuttle substrates that are misfolded or abnormal to the proteasome (262).

In some cases, mutant membrane proteins are dislodged from the membrane by Hsp90 and presented to the proteasome for degradation. For example, membrane-bound cytochrome *P*-450 2E1 (CYP2E1) is degraded by the proteasome dependent on Hsp90 (129). Hsp90 interacts both with CYP2E1 and the proteasome and promotes degradation of the former. Another important example is the degradation of CFTR. A large percentage of CFTR does not reach the plasma membrane due to improper folding. CHIP is a U box-containing ubiquitinating enzyme (E3/E4) and a cochaperone that interacts with Hsc70. Together, CHIP and Hsc70 recognize misfolded CFTR and promote its ubiquitination and degradation (303). CHIP also facilitates degradation of the glucocorticoid receptor by the proteasome along with Hsp90 (see sects. IV and V) (66). Furthermore, the proteasome can be found in specific cellular structures that include aggregated proteins, ubiquitin, and the cell stress chaperones Hsp70 and Hsp90, indicating that the proteasome is possibly targeted to concentrations of misfolded proteins that must be removed (421, 471). Even in vitro it seems that some substrates are proteolysed only with the help of chaperones (29), suggesting that "chaperone presentation" to the proteasome might be a feature of protein degradation (Fig. 6).

3. Alternative regulatory complexes

In addition to the 19S RP, another regulatory complex called the 11S REG or PA28 can activate peptidase activity of the 20S CP (92, 286, 423). PA28 increases maximum velocity for hydrolysis of certain peptides by the 20S CP by up to 100-fold, but as apposed to the 19S RP, it does not promote protein degradation by the CP (92, 286, 423). PA28 is a heteroheptamer or hexamer of two similar proteins, α and β, that can attach to either one or both outer α-rings of the 20S CP. This complex can be found in the cytoplasm of vertebrate cells, and cellular levels of both the α- and β-subunits of PA28 increase significantly by interferon-γ linking PA28 with the immune response (141, 364, 423). Additional related complexes to PA28/11S REG are also found in eukaryotes: REGγ (also called the Ki antigen or PA28γ) is a homo-heptamer found in numerous metazoans (even those lacking an adaptive immune system), and a more distantly related heptamer ring called PA26 is found meanwhile only in *Trypanosoma brucei* (362, 485). *S. cerevisiae* appears to lack homologs of this class of proteasome activators; however, seeing as PA28 and PA26 are highly divergent, it is possible that functional homologs with only low-level sequence similarity are present in yeast or additional eukaryotes.

Based on the crystal structure of a complex of PA26

from *T. brucei* with a 20S CP, PA26 attaches to the α-ring of the CP (469). The PA26 heptamer binds to each of the 20S CP α-subunits at their H1 and H0 helices, which are near to their NH_2 termini and protrude into the center of the ring (469). Upon binding of PA26, the NH_2 termini of the different α-subunits are disordered and pulled into the central cavity of PA26 and away from the center of symmetry defining the α-ring. It is assumed that similarly to PA26, PA28 also activates the CP by binding to the α-ring surface and rearranging the blocking NH_2-terminal residues thus opening the gate and increasing the rate by which peptides enter the CP (469). However, because peptides are products of the CP, it is possible that PA28 also affects the rate at which peptides are generated by the CP and exit the proteolytic chamber. A recently described 30-kDa inhibitor of the proteasome, PI31, appears to compete with PA28 in binding to the α-ring (300, 495); however, its physiological role has not been discerned.

4. Transiently associated factors

Cellular proteins could also bind to the proteasome and fine tune its activity. They do so by linking the proteasome to other signaling pathways, or by aiding proteasome assembly and localization. One group of proteins that is found tightly bound to the proteasome or its subunits is factors that assist in proteasome assembly. In mammalian cells, a protein called p27 has been found associated with two of the proteasomal ATPases (S6′/Rpt5 and S10b/Rpt4) and has been proposed to aid in the assembly of the RP (5, 77). The exact role of this modulator is unclear, however, especially since its yeast homolog, Nas2, is nonessential (465). Another yeast protein, Ump1, is needed for maturation of 20S CPs from α- and β-subunits and is degraded upon completion of assembly, in effect becoming the proteasome's first substrate (359).

Another class of transiently associated proteins is auxiliary factors that either shuttle substrates to the proteasome or target the proteasome to substrates. The proteasome interacts with components of the cell cycle machinery (216), and proteasomal subunits colocalize in vivo together with heat shock proteins and chaperones at sites of misfolded AR aggregates (213, 421). p28 has been shown to interact with the proteasome in mammals (182). p28 has five or six ankyrin repeats that can serve to promote protein-protein interactions. p28 is identical to the oncogene gankyrin that is overexpressed in certain carcinomas, binds retinoblastoma (RB), and promotes degradation of RB via its interaction with the proteasome (74, 168). Similar to a number of other "loosely associated" proteasomal proteins, Nas6, the yeast homolog of mammalian p28, is nonessential (182). Because many substrates of the proteasome contain ankyrin repeats, for example, IκB and certain cyclins, it is possible that p28 is either a substrate of the proteasome itself or a substrate shuttle that can aid in the recognition and anchoring of such substrates to the regulatory particle of the proteasome (74, 168).

Attachment of auxiliary factors and transiently associated factors to the proteasome occurs in response to specific signals. A number of proteins specifically associate with the proteasome during different developmental stages, or under different physiological conditions. For instance, in yeast, Nob1 cofractionates almost exclusively with the proteasome but only in growing and dividing cells, while the levels of Nob1 in stationary or resting cells are below detection (437). Because Nob1 is an essential gene, it would seem that Nob1 plays an essential role in regulating proteasomal function in growing cells (437). A blast search indicates that proteins with varying levels of homology to Nob1 are present in the genomes of numerous archaea and eukaryotes. In budding yeast, the protein Son1/Ufd5/Rpn4 can copurify with the proteasome by gel filtration or immunoprecipitation, although it is not found in purified preparations (103, 112, 127). However, because Rpn4 has been found to be a transcription factor that acts as a trans-activator of proteasome and ubiquitin pathways genes, Rpn4 is not an integral component of the proteasome, and its interactions with the proteasome are most likely due to its being a substrate (201, 292, 480). The nuclear protein HEC has also been shown to bind to MSS1 (the mammalian homolog of Rpt1) and inhibit its ATPase (59). It is possible that other proteins could also fine tune the function of the proteasome in relation to specific substrates.

5. Viral proteins

Interestingly, certain viral proteins such as HIV Tat or hepatitis B virus X protein (HBX) also interact with and inhibit the proteasome (186, 400, 499). HIV Tat not only inhibits the peptidase activity of the 20S CP, but also interferes with binding of PA28 to the CP (400). Thus one mechanism by which viruses can hamper antigen presentation is to decrease the generation of peptides by the proteasome. This could be achieved by maintaining the gate in a closed conformation. Indeed, Tat is a potent immunosuppressive agent (456). It is possible that this is a general mechanism by which certain viruses try to deceive the immune system, since other viral proteins, such as HBX, also interact with and inhibit the proteasome (186, 499).

The effects that viral proteins have on the proteasome can be quite complex. For instance, not all viral proteins seem to have only inhibitory affects on the proteasome. While HIV Tat inhibits peptide generation by the 20S, elevated rates of protein degradation by the 26S proteasome are found in the presence of Tat (400). Likewise, the viral oncoprotein E7 interacts with the Rpt2 ATPase of the RP and enhances its ATPase rate (30), possibly as a means to target degradation of RB by 26S proteasome (43). A confusing matter still is that E7 itself can be ubiquitinated and targeted to the proteasome for degradation (365).

E. Homology and Similarity to Other Cellular Complexes

1. 20S and other self-compartmentalized proteases

Archaea and some bacteria contain stripped-down versions of the proteasome, which play a regulatory role in the stress response (78, 298, 503). Ubiquitin or the ubiquitin-conjugating system are not found in these species; therefore, targeting of substrates to these proteasomes probably entails direct recognition of unfolded or damaged proteins. Prokaryotes contain a number of different compartmentalized protease complexes such as Lon/La, ClpAP, ClpXP, and FtsH, which are regulated in an energy-dependent fashion, although their subunits are genetically unrelated to proteasome subunits from eukaryotes (78, 137, 284). Bacteria do contain an ATP-dependent protease, HslVU, with a core protease ring (HslV) that shares significant homology to the β-subunits of the CP from eukaryotes. HslV can associate with a ring of ATPases (HslU) to form an ATP-dependent protease complex; however, HslU is not an Rpt-like ATPase, but rather related to the bacterial ClpX ATPases (373). Possibly, HslVU represents a dead end in the evolution of the proteasome from a CP-like protease. Proteasomal CP-like structures have been isolated from several archaea, and prokaryotes; however, it is unclear whether in all cases they also contain analogs of an RP (22, 69, 78, 297). However, the proteasome is nonessential in these organisms since they contain multiple ATP-dependent complex proteases with redundant functions, such as the Lon protease, ClpA/P, HslV/U, and the proteasome (78, 298, 503).

2. The base and other regulatory rings of ATP-dependent proteases

The proteasome is among the most complex ATPase assemblies to be described since six different ATPases reside within a single regulatory particle. The presence of six different ATPases within a given proteasome suggests that they may form a six-membered ATPase ring structure, analogous to those found in the homomeric ATPase regulatory rings of proteasomes from prokaryotes or archaea (476, 477, 504), or the ATP-dependent ClpAP and HslVU proteases (224, 373). The six different eukaryotic Rpt subunits may have diverged from a small number of evolutionary precursor ATPases, similar to the divergence of the eukaryotic CP's 14 subunits from a single α- and a single β-precursor found in the CP of archaea (37, 458).

The single *RPT* homolog in *Methanococcus jannaschii* (PAN) or *Rhodococcus erythropolis* (ARC) can form homo-hexameric rings (476, 477, 504). Moreover, the hexameric ring of PAN, perhaps the most rudimentary form of the RP, can regulate at least some functions of the archaeal 20S CP and confer upon it the capability to proteolyse certain nonubiquitinated proteins (476, 504). PAN rings do indeed attach to the outer surface of the α-ring of the CP (476). A distinction between proteasomes from archaea and yeast, however, is that the putative regulatory ring of ATPases from archaea does not enhance peptidase activity (504), whereas both the RP and the RP base from yeast do (126, 127). This may reflect a greater need for gating the channel leading into the proteasome from eukaryotic sources due to the asymmetric arrangement of the CP's seven different α-ring subunits (142, 143, 278). PAN has been shown to unfold proteins prior to their degradation by the 20S CP (318a).

A complex that is not obviously related to the base but which nevertheless deserves mention here is the valosin-containing-protein (VCP; also known as p97 or Cdc48). VCP/Cdc48 is a member of the AAA family and forms a hexameric ring. The archaeal homolog VAT has unfoldase activity (130a). VCP/Cdc48 is intimately linked with the Ub-proteasome pathway for degradation. For instance, VCP interacts with the U-box E3/E4 ligase Ufd2 and aids in degradation of a UFD multimeric substrate (241). Cdc48 together with Ufd1 and Npl4/Hrd4 also participates in ER-associated degradation (ERAD) by extracting proteins from the ER and targeting them to the proteasome (358a, 487a). This same complex also mediates proteasome-dependent membrane-bound transcription factor activation by removing processed and ubiquitinated p90 from its nonubiquitinated full-length partner Spt23 (23a, 171a, 360a). Remarkably, VCP/Cdc48 has been reported to bind polyubiquitin chains and polyubiquitinated substrates as well as the proteasome (69a). Thus VCP/Cdc48 could play a role in extracting ubiquitinated subunits from membranes or multimeric complexes for presentation to the proteasome. Whether VCP/Cdc48 is an ersatz regulatory particle in ERAD, substituting some of the functions of the 19S RP, or whether it participates in ubiquitin-substrate recognition for later transfer to the proteasome is still a matter for speculation.

The modular structure of the RP probably arose concomitantly with the advent of small protein labels such as ubiquitin, which are apparently absent from prokaryotes. For instance, while free CP can hydrolyze peptides, addition of either the eukaryotic ATPase-containing base of the RP, or an archaeal regulatory ring of ATPases (PAN), allows for proteolysis of non-ubiquitinated proteins (126, 504). Further attachment of the lid to the RP then allows for ubiquitinated protein degradation (126).

3. The lid, the COP9 signalosome (CSN), and eukaryotic initiation factor 3

The eukaryotic initiation factor 3 (eIF3) from human is a 600-kDa subcomplex that contains roughly 10 subunits, binds to the ribosome, and serves as an organization locus for the binding of mRNA and other proteins that trigger the initiation of protein translation. The yeast version of eIF3 is somewhat smaller and contains fewer

subunits. Both the human and yeast eIF3 complexes include subunits with PCI and MPN motifs, as well as proteins with homology to a specific lid component, S12/Rpn8 (17, 177). The COP9 signalosome (CSN) is a highly conserved complex that plays a role in regulating the development of eukaryotes (54, 80, 225, 396a, 400a). For instance, it is essential for light signal transduction in plants (55) and for development of *Drosophila* embryos (108). The CSN has been purified from a number of metazoans as a 450-kDa complex and is probably part of a larger, as yet unidentified, complex (401, 402, 467). Like the lid of the proteasome, the CSN is also an eight-subunit complex, six of which share a PCI domain, while the other two share an MPN domain (80). A number of CSN subunits have also been identified in *S. pombe*, indicating that the CSN plays a role in single cellular organisms (314).

Both the lid and the CSN contain the same number of subunits, have a similar molecular weight and architecture, and share the same structural motifs, and each subunit in each complex has a specific paralog in the other (80, 126, 221). The molecular arrangement and pairwise interactions between the synonymous subunits in the two complexes are remarkably similar if not identical (109a). In addition, these two complexes have similar intracellular distributions. Both particles were found to be concentrated at the nuclear periphery, but also present in the cytosol and the nucleus (97, 401, 472). Remarkably, these similar complexes have different biological roles. Several biochemical functions are associated with the CSN, either directly or indirectly, including phosphorylation of various regulatory proteins (24, 318, 401) and a hydrolase activity that removes Nedd8/Rub1, a modifier related to ubiquitin, that can be attached to the SCF E3 ubiquitin ligase complex (285, 397).

Although its exact functions are not yet known, the CSN and ubiquitin pathways seem intimately linked. Many CSN substrates are also substrates of the proteasome. These include transcription factors such as members of the AP-1, IRF, NF-κB, HY5, or Id families, cell cycle factors such as p53 or $p27^{kip1}$, and receptors such as the progesterone receptor (PR), the steroid receptor coactivator 1 (SRC-1), and the lutropin/choriogonadotropin receptor precursor (rLHR) (24, 40, 41, 56, 80, 271, 332, 407, 436). Indeed, reports suggest interactions between subunits of the proteasome and CSN (223, 248) or between CSN and the SCF ubiquitin ligase complex (285, 397). The link between the ubiquitin and CSN signaling pathways is also evident from the partnership between the CSN and the putative ring finger-containing E3, COP1, in regulating the degradation of the light-responsive transcription factor HY5 in plants (332). Furthermore, mutants of COP1 exhibit identical phenotypes to mutants of CSN subunits. These examples suggest that the CSN cooperates with the 26S proteasome in regulating protein degradation. It is possible that the shared homology and common architecture of the proteasome lid and the CSN are major features in their ability to share substrates.

CSN5, previously described as JAB1 (JUN activating domain binding protein 1), is the CSN subunit homologous to Rpn11 from the proteasome. It was identified genetically as a coactivator of JUN-mediated gene expression. Interaction of CSN5/JAB1 with c-*Jun* and JunD enhances specific c-*Jun*- or JunD-associated AP-1 activity by promoting c-*Jun* phosphorylation and increasing c-*jun* stability (63, 318). JAB1 was also shown to promote degradation of the CDK inhibitor $p27^{Kip1}$, probably causing its translocation from the nucleus to the cytoplasm, where it is degraded by the proteasome after phosphorylation and ubiquitination (see sect. IV). MIF is a cytokine with anti-inflammatory properties and negatively affects JAB1. While JAB1 suppresses $p27^{Kip1}$, MIF causes a reversal in CSN5/JAB1 activity, leading to CSN5/JAB1-dependent increase in $p27^{Kip1}$ (235). CSN5/JAB1 has been shown to bind to the immature precursor of the rLHR that resides in the ER and to promote its degradation by the proteasome (271). CSN5/JAB1 also enhances the hormone-dependent activity of the PR by stabilizing the complex of PR with SRC-1 (56). SRC-1 is a nuclear receptor/coactivator and a substrate of the proteasome in the absence of ligand (56, 407, 421). An additional feature associated with CSN5/Jab1 is hyperaccumulation of a Neddylated form of the Cullin-1 component of the SCF complex (or Rub1-Cdc53 conjugates in *S. cerevisiae*) found in absence of Csn5/Jab1 (285, 397).

The CSN or its subunits seem to have a direct effect on the stability and potency of specific members within a number of transcription factor families. In this manner, the interaction of CSN with certain transcription factors alters their ability to heterodimerize with their various partners. In a number of cases, this also has a direct affect on the stability of these transcription factors. For instance, the Jun proteins dimerize with the Fos proteins to form the AP-1 transcriptional activator. Each type of heterodimer activates a set of only partially overlapping AP-1 target genes. CSN5/JAB1 interacts with c-Jun and JunD, but not with JunB or v-Jun, and enhances the specific c-Jun- or JunD-associated AP-1 activity (63). Unphosphorylated c-Jun is normally an unstable protein that is rapidly removed from the cell by the proteasome (443). Interaction of the CSN with c-Jun increases its stability and elevates AP-1 activity, probably by promoting c-Jun phosphorylation (318, 401). Thus, through selective interactions with the Jun proteins, the CSN can increase the specificity of target-gene activation. A similar mechanism seems to regulate Id activity. The four Id proteins are a family of negative regulators of transcription that heterodimerize with transcription factors and prevent them from binding to their target DNA. Most Id proteins, as well as many of their partners such as MyoD and E12, are substrates of the proteasome and are rapidly degraded under certain conditions (40).

CSN5/JAB1 interacts with Id3, possibly affecting its stability or that of its partners (40).

Another example highlights the possible link between IRFs and the CSN. The CSN binds to IRF-8/ICSBP via its CSN2 subunit and promotes phosphorylation on a unique serine residue, thus enhancing the ability of IRF-8 to bind to IRF-1 (64). This dimerization protects IRF-1, an otherwise unstable protein, from degradation (see sect. IV). Finally, degradation of the light-responsive transcription factor HY5 in plants is mediated by the putative E3 enzyme COP1 (332). CSN mutants cause stabilization of HY5; it accumulates in the nucleus, and light-regulated genes are transcribed even in the dark.

From these examples, a pattern evolves in which the CSN presents, or prevents the presentation of, certain substrates to the proteasome. It is tempting to speculate that the shared homology and common architecture of the proteasome lid and the CSN are major features in their ability to share substrates. Given the fact that the proteasome and the CSN have common substrates and the homologies described between subunits of the two complexes, we propose that they may have common substrate binding sites and maybe complement each other's function.

VII. SITES OF INTRACELLULAR PROTEIN DEGRADATION

The ubiquitin-mediated system was thought initially to target only cytosolic proteins. Recent evidence indicates however that substrates for ubiquitination and subsequent degradation are found throughout the cell, in the cytosol, nucleus, ER lumen and membrane, and cell surface membrane (38, 46, 166, 279, 353). The two processes, degradation of cell surface and ER proteins, appear to be distinct and utilize different components. While ER proteins are transported in retrograde to the cytosol, where on the cytosolic face of the ER membrane they are ubiquitinated and degraded by the proteasome in the cytosol, the cytosolic tails of cell surface proteins are ubiquitinated, in many cases after ligand binding. They are then transported, via a series of endosomal vesicles, to the lysosome/vacuole where they are degraded. As for nuclear proteins, some are probably degraded in the nucleus, whereas for others it was reported that they have to be exported from the nucleus and are degraded in the cytosol. For these proteins, it is not clear whether ubiquitination, or at least partial ubiquitination, can occur in the nucleus. The majority of proteasomes, both the CP and RP subcomplexes, are localized proximal to the nuclear envelope (NE) and ER or to the nuclear core complexes (47, 97, 432, 472), suggesting that a significant portion of degradation of proteins may occur near the face of these organelles (see below).

A. ER-Associated Degradation

Normally, ER membrane proteins or proteins that cross the ER membrane are either retained in the ER or traverse the ER lumen en route to their final destination, the Golgi apparatus, cell surface membrane, extracellular environment, and the lysosomal/vacuolar system. However, abnormal/misfolded proteins or excess of unassembled subunits of multisubunit complexes are ubiquitinated and degraded in the outer/cytosolic leaflet of the ER membrane or in the cytosol after their retrograde transport. This mechanism serves an important role in cellular defense and quality control. Corroborating this notion are the findings that inhibition of ERAD elicits a series of stress responses known as the unfolded protein response UPR (109, 442) and that accumulation of undegraded denatured/misfolded proteins, probably in aggresomes, leads to inhibition of the ubiquitin system with potential severe consequences (25). Normal membrane proteins are also targeted by the system in a similar mechanism. Among the proteins degraded via ERAD are mutated model yeast proteins (169), the yeast (147) and human 3-hydroxy-3-methyl-coenzyme A reductase (HMGR) (361), the mutant Prion protein (90, 205), normal and mutated CFTR (243), the Wilson disease Cu^{2+} transporter protein (339), and MHC class I molecules targeted for degradation by the cytomegalovirus-encoded proteins US2 and US11 (405) (see below). Although the degradation pathway of these proteins traverses the "canonical" E1/E2/E3/proteasome route, it is nevertheless equipped with unique components essential for the retrograde transport. One is obviously the Sec61 translocation channel/pore through which the proteins are translocated back into the cytosol (352). Two E2 enzymes were identified that are essential for ERAD. One is Ubc6p that is a membrane-anchored protein, and the other is Ubc7 that is membrane-associated E2 (416). The active site Cys residues of the E2s are obviously facing the cytosol. Cue1 is an ER membrane protein necessary to recruit Ubc7 to the membrane (35). Interestingly, the degradation of Ubc6 itself, which is membrane anchored but not a membrane-spanning protein, does not require the Sec61p apparatus (460).

It has been demonstrated recently that Hrd1/Der3, also a membrane protein, is the ubiquitin ligase involved in HMGR degradation in yeast. It acts along with the E2 Ubc7, but also with Ubc1 (23). An important ERAD component is the yeast chaperone Kar2 (352) and its mammalian homolog BiP (205), and also the Hsc70 cochaperone CHIP (303) that appears to be an E3 that acts along with chaperones to ubiquitinate misfolded proteins (see above). The chaperones that reside on the luminal face of the ER membrane are probably involved in unfolding of the target proteins that enables their insertion into the translocation pore from the luminal side. Sec63 is a mem-

brane-binding partner of Kar2; however, its role in ERAD has not been discerned (352). The role of other proteins identified via genetic screen as ERAD components, Der1, for example (148) has not been elucidated either. Degradation of ER membrane proteins requires metabolic energy not only for activation of ubiquitin and activity of the proteasome, but probably also for the active retrograde transport. For instance, another complex with chaperone-like activities, VCP/Cdc48-Ufd1-Npl4, is involved in ERAD by extracting ubiquitinated proteins from membranes with subsequential presentation to the proteasome (358a, 487a).

B. Degradation of Cell Surface Proteins

G protein-coupled receptors, such as the α- (Ste2) and a- (Ste3) pheromone factor receptors, and transporters such as the uracil (Fur4) and maltose (Mal61) permeases in yeast, are modified with ubiquitin after ligand binding. Similarly, several mammalian cell surface receptors, many of them for growth factors, the growth hormone receptor, the platelet-derived growth factor receptor and the EGF-R, for example, undergo a similar modification in response to ligand binding. The kidney epithelial Na^+ channel (ENaC) is also modified by ubiquitin, although the underlying stimulus has not been clarified (166). In most cases, these proteins are not targeted by the proteasome. Rather, ubiquitination appears to activate the internalization and endocytic machineries that route the internalized proteins to the vacuole/lysosome, wherein they are degraded. This major difference in targeting distinct classes of ubiquitinated substrates to different destinations and proteolytic machineries implies probably a difference in the ubiquitinating enzymes and the type of ubiquitin modification that these proteins undergo. The mechanisms by which ubiquitin triggers internalization are still unknown. It is known however that for the α-factor receptor that undergoes mostly mono- and diubiquitination, modification by a single ubiquitin moiety is sufficient to trigger endocytosis (433). The same is true for the a-factor receptor (72).

Recent studies have shown that monoubiquitin is sufficient to serve as an internalization signal (167). Fusion of ubiquitin in-frame to the stable plasma membrane protein Pma1 as well as to the α-factor receptor stimulates endocytosis of these proteins. Although ubiquitin does not carry a tyrosine- or dileucine-based internalization signal, the three-dimensional structure of folded ubiquitin carries an internalization signal that consists of two surface patches surrounding the critical residues Phe-4 and Ile-44 (410). Degradation of these receptors occurs in the vacuole, as cells that are defective in vacuolar proteases but not in the proteasome display impaired degradation. The uracil permease appears also to be modified by oligo-ubiquitin chains, but the ubiquitin moieties are linked via Lys-63 (see above), a linkage that appears to be necessary for maximal rates of endocytosis (116). Here, however, the researchers have shown that polyubiquitination is important, and in cells that overexpress ubiquitin mutants that cannot generate a chain, endocytosis is slowed down markedly. The E2 enzymes involved in endocytosis of the α-factor receptor are Ubc4 and Ubc5 (375). These conjugating enzymes are involved in degradation of abnormal and short-lived proteins and in proteolysis in stressed cells (403) and are clearly distinct from Ubc6p and Ubc7p involved in degradation of ER proteins. As for the E3 involved, it has been shown that activity of the HECT domain ligase Rsp5p is required even for internalization of the ubiquitin-containing (fused) α-factor receptor, suggesting that the ligase is required for modifying a certain protein(s) that acts in *trans*, in addition to its probable role in modifying the receptor itself (93). For the growth hormone receptor it was demonstrated that ubiquitination is not essential for its endocytosis, although it is stimulated by ligand engagement, as substitution of all the Lys residues in the cytosolic tail did not affect the process (139). Yet, the ubiquitin conjugation machinery was still essential for endocytosis, and a motif in the tail designated the UbE (ubiquitin endocytosis) motif was required for this activity. Thus the tail appears to act in *trans*, in a yet to be revealed manner, in ubiquitination of a yet unknown factor that is required for growth hormone receptor routing.

For degradation of the kidney epithelial channel (ENaC), it is clear that polyubiquitination is necessary, and the E3 involved was identified as the HECT domain protein NEDD4 (376). Interestingly, the Nedd4 enzyme itself is regulated by phosphorylation. In this case, the phophorylation decreases the affinity between the enzyme and the channel leading to increased activity of the channel. This mechanism explains part of the effect of aldosterone on ENaC activation, as the hormone is known to induce the kinase Sgk1 that modifies the enzyme (75a). Likewise, the PDGF receptor is also multiply ubiquitinated before its degradation (312a). Thus, from the few examples reviewed, it appears that different membrane proteins use different ubiquitination machineries; however, they are all targeted to the lysosome/vacuole, and their polyubiquitin chains, when formed, do not appear to be targeted by the proteasome. An important unsolved problem involves the mechanisms by which ligand binding stimulates ubiquitination. An additional major, yet unresolved, problem in this context is the identity of the mechanism(s) that underlie the activity of ubiquitin, once it is conjugated, as an internalization signal. One possibility is that the ubiquitin chain serves as an anchor to an adaptor protein(s) that links it to component(s) of the ingoing endocytic pathway. This hypothesis assumes that this putative protein(s) is protected from interaction with free ubiquitin or ubiquitin chains that are present in the cytosol, and it interacts only with membrane protein-anchored ubiquitin moiety. Another hypothesis is that ubiquitin, in a yet to be determined manner, facilitates movement of the conjugated

proteins to subdomains within the membrane from which endocytosis occurs preferentially.

C. Degradation of Nuclear Proteins

Because many of the enzymatic components of the ubiquitin system such as E1 have nuclear localization signals (149, 302) and have been identified in the nucleus (396), and because many of the substrates of the system, transcription factors, and tumor suppressors, for example, act in the nucleus, it was assumed that the system is also active in the nucleus. At least for p53, β-catenin, and $p27^{Kip1}$, it appears that the process is more complex. Leptomycin B, a drug that inhibits the CRM1 nuclear export transporter, inhibited almost completely MDM2- and E6-AP/E6-dependent degradation of p53 (107), suggesting that it has to exit the nucleus to be degraded. Further studies have shown that the RING finger domain of MDM2 and its human homolog HDM2 are essential to promote nuclear export of p53 (42, 120). Thus it was hypothesized that the ligase "chaperones" p53 from the nucleus to the cytosol. The requirement for the RING finger, the domain that recruits the E2, suggests that monoubiquitination of the substrate, or at least attachment of few ubiquitin moieties, occurs in the nucleus, but completion of the process, including proteasomal digestion, is carried out in the cytosol (253). Yet, other studies have shown that p53 can be completely degraded in the nucleus (480a), and it appears that the problem of nuclear-cytoplasmic shuttling and proteolysis of p53 has yet to be resolved. Similarly, it has been shown that degradation of β-catenin is dependent on shuttling of APC (adenomatosis polyposis coli complex, to distinguish it from the anaphase promoting complex involved in degradation of cell cycle regulators; see above) from the nucleus to the cytosol (159). APC is part of the degradation complex of catenin, although it does not have an E3 activity of its own.

The degradation of mammalian $p27^{Kip1}$ appears also to occur in the cytosol, after JAB1 (p38)-mediated nuclear export (436). In contrast, Far1, a bifunctional protein required to arrest cell cycle and establish cell polarity in yeast during mating, was shown to be degraded in the nucleus. It is targeted, after phosphorylation, by the Cdc4 F-box protein. The ligase is confined solely to the nucleus. Furthermore, in contrast to p53, inhibition of nuclear export of Far1 destabilizes the protein, whereas prevention of its translocation into the nucleus rendered it stable (36). Similarly, degradation of *Xenopus* p27 (60) and of MyoD (105) was also shown to occur in the nucleus, although, for MyoD, cytosolic degradation probably also occurs. For the Deg1 degradation signal of the transcriptional repressor MATα2, it has been shown that it confers nuclear degradation to a reporter protein (264). A reporter that is exported efficiently from the nucleus displays an extended half-life. However, in this case it has not been shown whether degradation occurs in the nucleus or whether a transit through the nucleus is required to recruit the E3 or an essential nuclear exporting component. From all these cases it is clear that both routing of the substrate and compartment-specific activity of components of the ubiquitin system play important roles in governing the stability of different proteins. The physiological significance, however, of regulation via nuclear-cytoplasmic shuttling is still a mystery.

VIII. RECYCLING OF UBIQUITIN AND DEUBIQUITINATING ENZYMES

Posttranslational modification of proteins by covalent attachment of ubiquitin is a reversible process. All known deubiquitinating enzymes (DUBs) are cysteine proteases that specifically hydrolyze the amide bond immediately after the COOH-terminal residue (G76). DUBs belong to a large and diverse family of enzymes. In *S. cerevisiae*, there are at least 17 different DUBs, none of which is encoded by an essential gene, suggesting that they harbor overlapping functions (9). Other eukaryotes contain an even greater number of DUB enzymes. Based on their molecular size, sequence homology, and active site residues, DUBS are categorized as UCHs (ubiquitin COOH-terminal hydrolyses) or UBPs (ubiquitin-specific proteases). UCHs are generally small enzymes (20–30 kDa) that remove short or flexible peptide chains from the COOH terminus of ubiquitin. UCHs, like all cysteine proteases, contain a catalytic triad in their active site comprised of Cys, His, and Asp residues, as well as an additional conserved Glu residue (61, 70). All four active site residues reside within a typical consensus sequence. *S. cerevisiae* genome encodes a single UCH, *YUH1*, while *S. pombe* has at least two, and other eukaryotes a larger number (61, 272). UBPs on the other hand belong to a larger and a more diverse group of enzymes and have a larger molecular mass, typically in the range of ~100 kDa. UBPs can cleave the isopeptide bond linking Ub-Ub or Ub-protein. In addition, they cleave also biosynthetic linear fusions of ubiquitin. UBPs also contain conserved cysteine, histidine, and aspartate residues within typical domains, although the consensus sequences around these active site residues can be quite divergent (70). Despite the common active site residues, the UBP and UCH families do not share sequence homologies with one another or with cysteine proteases.

DUBs play several roles both in maintaining the steady-state levels of free ubiquitin and in affecting the stability of Ub-conjugated proteins (61, 70, 474). These roles include the generation of ubiquitin, recycling of ubiquitin, editing polyubiquitin chains, and aiding in proteasome-dependent degradation. Ubiquitin is not expressed directly as free ubiquitin (i.e., there is no ORF corresponding precisely to the se-

quence of a single ubiquitin moiety), but rather as linear fusions either to itself (e.g., the *UBI4* gene in yeast), or to certain ribosomal protein subunits. Upon expression, these fusion precursors are rapidly processed by an as yet unidentified DUB generating free ubiquitin molecules (Fig. 6; arrow 12). Ubiquitin is also recycled into the ubiquitin system by DUBs that remove chains from substrates and/or disassemble chains (Fig. 6; arrow 3). Two examples are Ubp4/Doa4 (yeast) or Tre-2 (human) that remove ubiquitin from short peptides or proteasomal degradation products (335, 336, 427) and Ubp14 (yeast) or Iso-T (mammals) that disassemble unanchored polyubiquitin chains into monomeric ubiquitin (10, 145, 475) (Fig. 6, arrow 12). Mutations in Ubp4/Doa4, for example, cause improper degradation of the yeast MATα2 mating factor (427) or of the uracil permease (116). In the case of MATα2, the cells accumulate polyubiquitinated MATα2 fragments. This is the reason that both Ubp4 and Ubp14 are thought to promote proteasome-dependent protein degradation by recycling ubiquitin and maintaining proper levels of free ubiquitin in the cell.

A number of reports indicate that the RP of the proteasome has a deubiquitinating (DUB) activity (98, 174, 254, 256, 259, 272). The *Drosophila* DUB p37a (174) and its homologs UCH37 (*Homo sapiens*) and Uch2 (*S. pombe*) may be responsible for the polyubiquitin chain editing function associated with proteasomes (174, 254, 272). In *S. cerevisiae*, which lacks an obvious ortholog of p37a, Doa4 that interacts weakly and substoichiometrically with the proteasome, may serve to release ubiquitin and regenerate the proteasome for the next catalytic cycle (335).

A neuron-specific UCH, Ap-uch, promotes proteasomal degradation by removing polyubiquitin chains from substrates during, or after, 20S CP proteolysis (155). Apuch is involved in long-term memory by promoting the degradation of the inhibitory, R, subunit of the cAMP-dependent PKA. Enhanced PKA activity that follows removal of the R subunit allows probably for the transcriptional activity necessary for long-term facilitation and growth of new synapses. BAP1 is a nuclear UCH that serves as a tumor suppressor in the BRCA1 growth control pathway (202). BRCA1 is a RING finger domain E3 enzyme and can interact physically with BAP1. A UBP termed fat facets is necessary for differentiation in *Drosophila* eye cells (51, 194). Mutants in *D. melanogaster* fat facets show defects in the number of cells in the eye omatidium (188, 189). In all of the above cases, the cellular levels of a subset of ubiquitinated proteins are probably altered, underlying the importance of different DUB enzymes for normal physiological functions.

IX. UBIQUITIN-LIKE PROTEINS

Posttranslational modification of proteins by other proteins is not unique to ubiquitination. Quite a few small molecules (at least 11 have been described to date) can be covalently conjugated to amino residues of other proteins. These proteins are often called ubiquitin-like proteins (UBLs) even though some show very little resemblance to ubiquitin. In all cases, the modes of conjugation and sites of attachment to the substrate resemble modification by ubiquitin. UBLs are divided into two groups: *1*) those that are free-standing moieties that can be conjugated posttranslationally to other gene products and *2*) those that are synthesized as fusion proteins. The first group of proteins can posttranslationally form an isopeptide bond with an internal lysine of a substrate, just as ubiquitin does. No modification by poly-UBL chains is known. Members of the second class are larger proteins that contain a UBL domain; one can consider this as a UBL domain that is fused via a peptide bond to the remainder of the protein.

1) This group includes small proteins (76–103 residues) that modify other proteins posttranslationally. These include the SUMO/Sentrin/Smt3p family of proteins and NEDD8/Rub1; both these proteins show sequence homology to ubiquitin (~20 and 60% identity to ubiquitin, respectively). APG12, URM1, and HUB1 are unrelated by sequence homology to ubiquitin but are often referred to as "UBLs" since they are small protein modifiers that are activated and conjugated to other proteins in a manner resembling ubiquitin. Another related protein is ISG15 that contains two UBL domains. Importantly, and similar to ubiquitin, almost all these proteins have a GG sequence at their COOH terminus. This sequence is usually exposed after posttranslational processing by a specific protease. It is this GG sequence that is necessary for recognition and activation by their specific activation enzymes (E1) (173, 204, 488).

Activation and conjugation of UBL proteins proceeds in an analogous manner to that of ubiquitin (see also sect. v). First, the gene product must be processed by a specific protease (akin to the deubiquitinating enzyme that releases free ubiquitin moieties from UBI4; see sect. vii) to expose the functional COOH-terminal GG sequence. The UBL is activated by an activating enzyme, "E1," which is typically a heterodimer made of two subunits, one homologous to the NH_2-terminal domain of E1 and the other its COOH-terminal domain. The activating enzyme for Sentrin/Sumo/Smt3 is the pair AOS1-UBA2 (134, 210), whereas that for NEDD8/Rub1 is the dimmer APP-BP1 (or Ula1/AXR1 with Uba3/Ecr1) (134, 273). A single activating enzyme, however, activates other UBLs: Apg7 activates the UBL Apg12, and Uba4 activates Urm1 (114, 237) (see sect. v). The UBLs are then transferred to an E2 enzyme, once again via a thiol-ester bond. The Sumo/Sentrin/Smt3 family of UBLs is conjugated by Ubc9/UBCH9, whereas Rub1/NEDD8 utilize Ubc12 (83, 134, 208, 273). Apg10 is the E2 for Apg12 (237). In some cases, the E2 most probably conjugates the UBL directly to a lysine side chain on the target substrate. In the case of SUMO, a specific E3 has been identified (208a).

There seems to be a link between modification of proteins by some UBLs and modification by ubiquitin. Most proteins modified by SUMO/Sentrin/Smt3 are found in the nucleus or nuclear envelope. One target is PML, a putative RING finger-containing E3 enzyme (218). After conjugation, PML is found mainly in the nucleus. Likewise, conjugation of SUMO/Sentrin to known substrates of the ubiquitin/proteasome pathway, p53, RanGAP1, SP100, and HIPK2, increases their levels in the nucleus compared with the cytoplasm (488). The Ran GTPase-activating protein RanGAP1 is highly concentrated at the cytoplasmic periphery of the nuclear pore complex (NPC), where it associates with the 358-kDa Ran-GTP-binding protein RanBP2. This interaction requires the posttranslational conjugation of RanGAP1 with SUMO-1 that serves to target RanGAP1 to the NPC (289, 305). Another example that highlights the connection to the ubiquitin system is modification by SUMO-1/Sentrin-1 of IκBα, which precludes its ubiquitination and degradation as it occurs on Lys-21 or Lys-22 that serve as the polyubiquitin chain anchors as well (83) (see sect. IV). An interesting case involves sumoylation of Mdm2. Mdm2 is an E3 ubiquitin ligase for the p53 tumor suppressor protein. It is conjugated with SUMO-1 at Lys-446, which is located within the RING finger domain and reduces Mdm2 self-ubiquitination. It thus elicits increased degradation of p53 accompanied by concomitant inhibition of p53-mediated apoptosis. Radiation results in a decrease in the degree of Mdm2 modification, which is inversely correlated with the levels of p53. Thus it appears that the maintenance of the intrinsic activity of a RING finger E3 ubiquitin ligase is sumoylation dependent and that reduced Mdm2 sumoylation in response to DNA damage contributes to p53 stability (49).

The link between Rub1/NEDD8 to the ubiquitin pathway is clearer. The major target of Rub1NEDD8 is Cdc53/Cul-1 that is one of the components of the SCF E3 ubiquitinating complex. Even though *RUB1* is nonessential in *S. cerevisiae*, the NEDD8 system in mammals is essential as is the modification of Pcu1 (the *S. pombe* homolog of cdc53/Cullin 1) by NEDD8 (330, 432a). Conjugation of Rub1/NEDD8 to Cdc53/Cul-1 seems to stabilize the interactions of the latter with E2s such as Cdc34 or Ubc4, and at the same time to a variety of F-box proteins (219, 223a, 273). Furthermore, Neddylated Cul-1 stimulates the ability of the participating E2, Cdc34, to catalyze multi-ubiquitin chain assembly (477a). As the CSN is involved in decreasing the levels of Rub1/NEDD8 conjugation to Cdc53/Cul-1 (285, 501a), it might play a general role in downregulating SCF activity.

The UBL modifier Apg12, conserved in all eukaryotes, is conjugated to a lysine residue of only one known target, Apg5. This conjugated protein plays an essential role in autophagosome vesicle formation and delivery of cytoplasmic and possibly endocytosed/pinocytosed material to the vacuole (114, 237).

2) The second class of UBLs contains proteins with a distinct ubiquitin-like domain within a larger protein. This is a heterogeneous group, with the only common denominator a region within the protein that shows sequence homology to ubiquitin. Despite the resemblance to ubiquitin, the ubiquitin-like domain does not in any of these examples end with a Gly-Gly sequence, the UBL domain is permanently fused, and these proteins are not processed by DUB-like proteases (204). Nevertheless, many of these UBL fusion proteins are intimately involved with the ubiquitin pathway. Members of three groups within this family, Rad23/HHR23A/HHR23B, Dsk2/hPLIC1/hPLIC2, and Bag-1, can bind to the proteasome (172, 236, 257, 282, 329) (see sect. VI). It is possible that there is a specific UBL-binding site in the proteasome that may or may not be shared with the polyubiquitin recognition receptor. Binding of specific UBL domain-containing proteins seems to link the proteasome with potential substrates, whether misfolded proteins (via Bag-1) or sites of DNA damage and repair (via Rad23) (282, 380) or with ubiquitin ligases (hPLIC) (236), or polyubiquitinated substrates (56a, 471a). Additional UBL domain-containing proteins are also linked to the ubiquitin system. Ubp6 is a UBL-containing DUB enzyme, whereas Parkin and UIP28 are E3s. It is not clear yet what role their UBL domain plays in their interaction with their substrates or with the proteasome.

X. UBIQUITINATION IN THE PATHOGENESIS OF HUMAN DISEASES

With the many processes and substrates targeted by the ubiquitin pathway, it is not surprising to find that aberrations in the system underlie, directly or indirectly, the pathogenesis of many diseases. Although inactivation of a major enzyme such as E1 is obviously lethal (see Fig. 2), mutations in enzymes or in recognition motifs in substrates that do not affect vital pathways or that affect the involved process only partially, may result in a broad array of phenotypes. Likewise, acquired changes in the activity of the system can also evolve into certain pathologies. The pathological states associated with the ubiquitin system can be classified into two groups: *1*) those that result from loss of function, mutation in a ubiquitin system enzyme or target substrate, that result in stabilization of certain proteins and *2*) those that result from gain of function, abnormal or accelerated degradation of the protein target. Some of these pathologies are described briefly. Studies that employed targeted inactivation of specific ubiquitin system enzymes and substrates coding genes in animals can provide a glimpse into the broad spectrum of pathologies that may result from aberrations in ubiquitin-mediated proteolysis.

A. Malignancies

Alterations in ubiquitination and deubiquitination reactions have been directly implicated in the etiology of

many malignancies. In general, specific cancers can result from stabilization of oncoproteins or destabilization of tumor suppressor genes. Some of the natural substrates for degradation by the ubiquitin-proteasome system are growth-promoting factors that if not properly removed from the cell can promote cancer. For instance, ubiquitin targets N-myc, c-*myc*, c-*fos*, c-*jun*, Src, and many cell surface receptors for growth factors such as the EGF-R. Destabilization of tumor suppressor proteins such as p53 and p27 has also been implicated in the pathogenesis of malignancies.

It was noted that the level of the tumor suppressor protein p53 is extremely low in uterine cervical carcinoma tumors caused by high-risk strains of the human papillomavirus (HPV). Detailed studies both in vitro and in vivo have shown that the suppressor is targeted for ubiquitin-mediated degradation by the HPV oncoprotein E6 coded by high-risk species of the virus (E6–16 or 18, for example; Ref. 390). Low-risk strains that encode slightly different E6 proteins (such as E6–11) do not transform cells and do not target p53 for degradation. Degradation is mediated by the HECT domain E3 enzyme E6-AP, where E6 serves as an ancillary protein that allows recognition in *trans* (see sect. IV). It associates with both the ubiquitin ligase and the target substrate and brings them, via the generation of a ternary complex, to the necessary proximity that is assumed to allow catalysis of conjugation to occur. Removal of the suppressor by the oncoprotein is probably an important mechanism used by the virus to transform cells.

Likewise, low levels of the cyclin-dependent kinase inhibitor of the cell cycle, $p27^{Kip1}$, which result from increased ubiquitin-mediated degradation, have been demonstrated in colorectal, prostate, and breast cancers (277). The protein acts as a negative growth regulator/tumor suppressor and plays an important role in proliferation of mammalian cells. It binds and negatively regulates the activity of CDK2/cyclin E and CDK2/cyclin A complexes and thus does not allow cell cycle progression from G_1 and entrance into the S phase. Its level is high in quiescent cells, but after mitogenic stimuli, it is rapidly degraded by the ubiquitin system, allowing the CDK/cyclin complexes to drive the cell into the S phase (409). As noted, the level of p27 is markedly reduced in several cancers, including breast, colorectal, and prostate carcinomas, and in many of these cases there was a strong correlation between the low level of p27 and the aggressiveness of the disease: tumor grading, clinical staging, and poor prognosis of the patients (414, 444). Levels of p27 have become an important prognostic factor for survival, recurrence, and evaluation of therapy, where extremely low expression predicts poor prognosis. Dissection of the mechanism(s) that underlies the decrease in p27 revealed that unlike the case of other tumor suppressors like p53 that are mutated and stabilized in many tumors, the rapidly degraded p27 is of the wild-type species, and it is probably abnormal activation of the ubiquitin system that leads to accelerated degradation of the suppressor. Detailed mechanistic analysis revealed that the low level of p27 correlates directly with increased level of Skp2, the F-box protein involved in p27 ubiquitination, and that ectopic overexpression of Skp2 in experimental animals is oncogenic (144).

β-Catenin plays an important role in signal transduction and differentiation of the colorectal epithelium, and aberrations in ubiquitin-mediated regulation of its levels may be important in the multistep development of colorectal tumors (307). These tumors develop in 50% of the Western world population by the age of 70, and in 10% of these individuals (5% of the population), the tumors progress to malignancy. Fifteen percent of these patients have a genetic predisposing defect leading to the development of the malignancy. In the absence of signaling, glycogen synthase kinase 3β (GSK3β) is active and promotes degradation of β-catenin via the ubiquitin-proteasome pathway (234). Stimulation promotes dephosphorylation, stabilization, and subsequent activation of β-catenin via complex formation with T cell factor (TCF) and lymphocyte enhancer factor-1 (LEF-1), otherwise inactive subunits of transcription factor complexes. β-Catenin interacts with the 300-kDa tumor suppressor APC (adenomatous polyposis coli) that appears to regulate, in a yet unknown manner, its intracellular level. Aberrations in degradation of β-catenin lead to its stabilization, accumulation, and subsequent oncogenic activation, and can result from two distinct mechanisms: *1*) mutations in the phosphorylation recognition motif of the protein (see above) and *2*) mutations in the targeting machinery. Mutations in the E3 recognition domain of β-catenin, in which target residues of GSK3β that undergo phosphorylation are substituted with amino acids that cannot be modified, have been described in several colorectal carcinomas, but also in malignant melanomas (378). These mutations result in a protein species that cannot be phosphorylated, and therefore cannot be recognized by the ubiquitin ligase. Colon cancer cells that do not express APC, or that harbor APC proteins that are mutated in one of the catenin binding clusters, do not associate with β-catenin. Here too, the protein accumulates in the cytosol and is translocated into the nucleus where it acts as part of an active transcriptional complex. Expression of full-length APC in these cells leads to degradation of excess β-catenin and to abrogation of the *trans*-activation effect. It is possible that APC, while not a ubiquitin ligase itself, serves in a mechanism similar to that of E6, as an ancillary targeting element in *trans* for β-catenin (355).

c-Jun, but not its transforming counterpart v-Jun, can be multiply ubiquitinated and rapidly degraded in cells. The stability and lifetime of v-Jun are therefore greatly enhanced, which can lead to malignant transformation. Mechanistic analysis of the differential sensitivity to the ubiquitin system revealed that the δ-domain of c-Jun, an

amino acid sequence that spans residues 31–57 and that confers instability upon the normal cellular protein, is missing in the retrovirus-derived molecule (443). Deletion of this domain stabilizes c-Jun. Interestingly, the δ-domain does not serve as the site for ubiquitination as it lacks lysine residues. It may serve, however, as an anchoring site to the specific Jun ubiquitin-ligase. The loss of the δ-domain during retroviral transduction is yet another example of the "sophisticated" diverse mechanisms evolved by viruses to ensure replication and continuity of infection. Although mutated Jun has been identified in several tumors, the mutation has not been implicated directly with the pathogenesis of any known malignancy.

Mutations in components of the ubiquitination machinery can also cause malignancies. Mutations in one germ line copy of *VHL* predisposes individuals to a wide range of malignancies, including renal cell carcinoma, pheochromocytoma, cerebellar hemangioblasomas, and retinal angiomas. A hallmark of $VHL^{-/-}$ tumors is a high degree of vascularization that arises from constitutive expression of hypoxia-inducible genes, including the master switch transcription factor hypoxia-inducible factor 1-α (HIF1-α) and the crucial vascular endothelial growth factor (VEGF). It has been recently shown that pVHL is a ubiquitin ligase (196, 276) that is involved in targeting of HIF1-α for ubiquitin- and proteasome-mediated degradation (219, 220, 299) after specific recognition of oxygen-induced hydroxylated Pro residue (195, 197) (see also sect. II). It is possible that the high, nonregulated activity of HIF1-α, and possibly other targets of pVHL, underlie the high frequency of malignant transformations observed in carriers of mutations in pVHL. Another oncoprotein is c-Cbl that attenuates cell proliferation by serving as an E3 for membrane tyrosine kinase receptors, the EGF-R for example (206, 269, 270). Mutant forms of Cbl cannot properly terminate signaling, causing disregulated cell proliferation (263).

The human oncogene *tre-2* has been shown to encode a deubiquitinating enzyme that disassembles multiubiquitin chains from peptides still bound to the proteasome (336). Because the ubiquitin pathway rapidly degrades many proto-oncoproteins, increasing the levels of such proteins by inactivating the deubiquitinating enzyme *tre*-2, for example, can potentially be tumorigenic. It should be noted however that no malignancies have been associated with or attributed to mutations in *tre-2*. Overexpression of another DUB of the UBP family, Unp, has been found in lung carcinoma in humans and in mouse tumors (140). Unp may have a role in regulating the degradation of specific, as yet unidentified, substrates in these tissues, although it does not seem to be crucial for general proteolysis (121).

Mutations in the machinery involved in monoubiquitination of the Fanconi D protein that is essential to its localization with BRCA1 in nuclear foci following DNA damage and for DNA repair, have been shown to be involved in the pathogenesis of Fanconi anemia. Affected patients display high sensitivity to mitogens and irradiation, which result in high frequency of malignancies (118).

B. Liddle's Syndrome

An interesting syndrome involves aberration in the regulation of kidney ENaC. The channel, which is composed of three homologous subunits α, β, and γ, is a short-lived protein that is degraded by the ubiquitin system and is recognized via a proline-rich (PY) motif by NEDD4, a member of the HECT domain family of E3s. Binding of NEDD4 is mediated via a WW domain in the ligase (376). A mutation in the PY motif leads to stabilization of the channel complex, as it does not associate anymore with the ligase, accumulation of its subunits, excessive reabsorption of Na^+ and H_2O, and consequently, a severe form of early-onset hypertension.

C. Angelman Syndrome

The ubiquitin system is probably involved in human brain development. A defect in the gene coding for the E3 ligase E6-AP has been implicated directly as the cause of Angelman syndrome characterized by mental retardation, seizures, out of context frequent smiling and laughter, and abnormal gait (229, 251). The target brain protein(s), which are most probably stabilized because of the mutation, has not been identified. However, unlike in E6-mediated targeting of p53, the degradation of this putative protein(s) is E6 independent, and it is probably one of the native substrates of E6-AP. HPV, during its evolution and as part of its replication and propagation "strategy," "adopted" the already existing native cellular enzyme for targeting p53 in an E6-dependent mode (see above). Interestingly, the disease displays genomic imprinting where a subset of mammalian genes can be expressed monoallelically in a parent-of-origin manner (uniparental disomy, UPD). The imprinting process epigenetically marks alleles according to their parental origin during gametogenesis. In the case of Angelman syndrome, the imprinted gene is localized to chromosome 15q11-q13 where the deletions in the disease were identified. Confirmatory studies in mice with UPD of the mouse homolog (Ube3a) show marked reduction in expression within Purkinje cells and hippocampal neurons, thus strongly establishing imprinting of brain E6-AP in Angelman syndrome pathogenesis (7).

D. Neurodegenerative Diseases

Accumulation of ubiquitin conjugates and/or inclusion bodies associated with ubiquitin, proteasome, and

certain disease-characteristic proteins have been reported in a broad array of chronic neurodegenerative diseases, such as the neurofibrillary tangles of Alzheimer's disease (AD), brain stem Lewy bodies (LBs), the neuropathological hallmark in Parkinson's disease (PD), LBs in LB dementia, Bunina bodies in amyotrophic lateral sclerosis (ALS), and nuclear inclusions in CAG repeat expansion (polyglutamine extension) disorders such as Huntington's disease, spinocerebellar ataxias (SCAs), and spinobulbar muscular atrophy (Kennedy's syndrome). However, in all these cases, a direct pathogenetic linkage to aberrations in the ubiquitin system has not been established. One factor that complicates the establishment of such linkage is the realization that many of these diseases, such as AD and PD, are not defined clinical entities, but rather syndromes with different etiologies. Accumulation of ubiquitin conjugates in Lewy inclusion bodies in many of these cases may be secondary and reflect unsuccessful attempts by the ubiquitin and proteasomal machineries to remove damaged/abnormal proteins. While the initial hypothesis was that inclusion bodies are generated because of the inherent tendency of the abnormal proteins to associate with one another and aggregate, it is now thought that the process may be more complex and involves active cellular machineries (99, 212, 213), including inhibition of the ubiquitin system by the aggregated proteins (25). This aggregation of brain proteins into defined lesions, although it is not clear whether they are at all toxic, is emerging as a common but poorly understood mechanistic theme in sporadic and hereditary neurodegenerative disorders.

The case of PD highlights the complexity of the involvement of the ubiquitin system in the pathogenesis of neurodegeneration. Recent evidence has implicated mutations in α-synuclein, such as A53T, in the pathogenesis of neurodegeneration. Initially, it was identified in several cases of autosomal dominant familial cases of PD. Later, the protein was shown to be a major component of LBs and Lewy neurites in sporadic PD, dementia with LBs (DLB), and the LB variant of AD. Also, studies of brains from patients with AD caused by genetic abnormalities demonstrated many α-synuclein positive LBs (246). The function of α-synuclein is not known. Furthermore, although it has been shown that the protein is targeted by the proteasome (27), it is not known whether the mutations affect its stability and lead to its accumulation and the resulting toxicity. Moreover, although LBs contain α-synuclein and ubiquitin, it is not clear that it is the conjugated form of synuclein that is accumulated. In a different case, recent findings in a German family with PD have revealed mutations in the gene encoding the UCH isozyme UCH-L1 (265). Although the mutation did not lead to complete inactivation of the enzyme, the enzyme was clearly less active then its wild-type counterpart. The hypothesis here is that the mutation results in shortage of free ubiquitin required for degradation of certain, yet unidentified, proteins, that when they accumulate, they become toxic to neurons. Interestingly, mutation in UCH-L1 leads to gracile axonal dystrophy (GAD syndrome) in mice. This is a recessive autosomal mutation that displays sensory ataxia at an early stage, followed by motor ataxia at a later stage (383). A third player in the pathogenesis of PD is Parkin. Parkin is a 465-amino acid residue protein with moderate similarity to ubiquitin at the NH_2 terminus and a RING finger motif at the COOH terminus. Mutations in the gene appear to be responsible for the pathogenesis of autosomal recessive juvenile parkinsonism (AR-JP), one of the most common familial forms of PD (232). Interestingly, this disease is characterized by lack of LBs, the pathognomonic hallmark of sporadic forms of the disease. Later studies have identified Parkin as a ubiquitin-protein ligase that acts along with the ubiquitin-conjugating enzyme UBCH7 and UBCH8 and have shown that mutant Parkins from AR-JP patients display loss of the ubiquitin-protein ligase activity (411, 498). Parkin ubiquitinates and promotes degradation of several proteins, among them is CDCrel-1, a synaptic vesicle-enriched septin GTPase implicated in the inhibition of exocytosis through its interactions with syntaxin (498). Whether aberrations in the degradation of these substrates or of another, yet unidentified, substrate(s) of Parkin underlies the pathogenesis of AR-JP is not known. Nevertheless, the discovery of Parkin, its general function, and its mutations in PD is of utmost importance, as these findings point clearly toward a direct etiological role of this enzyme. Thus three distinct pathologies and proteins, all linked one way or another to activity of the ubiquitin system, α-synuclein, UCH-L1, and Parkin, have been implicated in the pathogenesis of different forms of PD, although the underlying mechanisms are still obscure.

AD is also characterized by accumulation/association of ubiquitin with Tau in neurofibrillary tangles and senile plaques, both characteristic of the neuronal abnormalities associated with the disease. They are also present in LBs characteristic to some forms of the diseases. However, in all these cases, the role of Tau and other putative target proteins in the pathogenesis of the disease is still not known. Also, here too it is not clear whether aberration in proteolysis plays a causative role, or whether the system plays only a secondary role, being unable to degrade the mutated/misfolded proteins, the accumulation of which is toxic. Recent evidence indicates that the pathology may be more complex, and the accumulated proteins that are inefficient substrates also actively inhibit the ubiquitin system (25). A more direct relationship between the ubiquitin system and pathogenesis of AD was established with the recent discovery of a frameshift mutation in the ubiquitin transcript which leads to extension of the molecule with 20 amino acid residues [Ub(+1)] and which has been

selectively observed in the brains of AD patients, including those with late-onset, nonfamilial disease (451). Ub(+1) is an efficient substrate for polyubiquitination; however, the resulting polyubiquitin chains are refractory to disassembly by deubiquitinating enzymes (255) and potently inhibit the degradation of a polyubiquitinated substrate by 26S proteasomes. Thus expression of Ub(+1) in the brain could potentially result in dominant inhibition of the ubiquitin-proteasome system, leading to accumulation of toxic proteins with neuropathological consequences. Because Ub(+1) was described also in other disorders such Down's syndrome (451) or supranuclear palsy (101), it is clear that it is not entirely specific to AD, and a major problem of how the mutation leads to distinct pathologies in different patients remains unsolved. Additional and probably distinct players in the pathogenesis of AD are the presenilins (PS) 1 and 2 that are involved in proper processing of the amyloid precursor protein (APP). Numerous mutations causing early-onset AD have been identified in the PS genes, particularly the PS1 gene. Both PS1 and PS2 are targeted by the ubiquitin system (226, 420). It is not clear whether aberrations in the degradation of the mutant proteins play any role in the pathogenetic process, or it is an aberration in the function of the PSs in processing of APP that underlies the pathology observed in AD. Thus, similar to PD, different ubiquitin-related pathologies are observed in different AD syndromes.

Another group of genetically inherited neurodegenerative disorders is caused by genomic instability that leads to an expanded 5′-CAG repeat which is translated to a NH_2-terminal polyglutamine repeat extension. In the case of Huntington's disease (HD), the affected gene is *Huntingtin*, which codes for a protein with unknown function. Similarly, in a series of diseases known as spinocerebellar ataxias (SCAs) 1–3, the affected proteins are ataxins 1–3, respectively, whereas in spinobulbar muscular atrophy, the affected protein is the AR (274, 374). The polyglutamine-containing proteins aggregate and accumulate in intranuclear inclusion bodies that are stained also for ubiquitin that is probably attached to them (8, 274, 374, 408). Huntingtin (217) and ataxin 1 (68) are probably targeted by the ubiquitin system. Furthermore, at least for ataxin 1, it has been shown that the degradation of the polyglutamine-containing and mutated protein in vitro is somewhat slower than that of the wild-type protein (68), suggesting that the inability of the system to remove the mutated protein may underlie its accumulation and possible aggregation. Although it is clear that the known catalytic sites of the proteasome cannot cleave within the polyglutamine repeat, it is not known whether the aberration in the system leads to accumulation of the intact proteins or long polyglutamine stretches that are released from them. Also, it is not clear whether the aggregated protein and/or the inclusion bodies are toxic, and their generation underlies the pathogenesis of the disease. Initial experimental evidence suggests that the inclusion bodies may serve as an alternative scavenger mechanism to store proteins that cannot be removed by the ubiquitin system and that "solubilization" of these proteins via expression of chaperones or removal of E6-AP (their putative ligase), two manipulations that do not allow the proteins to precipitate in either their free or conjugated form, aggravate the disease symptoms (68). If the inclusion body scavenger hypothesis is correct, then their formation is protective rather than toxic.

E. Cystic Fibrosis

The cystic fibrosis (CF) gene encodes the CFTR that is a 1,480-amino acid residue chloride ion channel. Only a small fraction of the wild-type protein matures to the cell surface, whereas most of the protein is degraded from the ER by the ubiquitin system. Although more than 600 mutations have been described, the most frequent one (>70%) is ΔF508. The mutation leads to an autosomal recessive inherited multisystem disorder characterized by chronic obstruction of airways and severe maldigestion due to exocrine pancreatic dysfunction. Despite normal ion channel function, $CFTR^{\Delta F508}$ does not reach the cell surface at all and is retained in the ER from which it is degraded. It is possible that the rapid and efficient degradation results in complete lack of cell surface expression of the ΔF508 protein and therefore contributes to the pathogenesis of the disease (243).

F. Immune and Inflammatory Response

Peptide epitopes presented to CTLs on class I MHC molecules are generated in the cytosol from limited processing of antigenic proteins. It is now generally accepted that processing of most known MHC class I antigens is mediated by the ubiquitin-proteasome pathway (238, 370). An interesting finding is that the cytokine γ-interferon that stimulates antigen presentation also leads to induction and exchange of three proteasomal subunits in human cells that lead to alteration in the cleavage site preferences of the proteasome (see above). The changes in activities probably result in peptides that terminate mostly with basic and hydrophobic residues, similar to the vast majority of known peptides presented on MHC class I molecules and bound to the T-cell receptor. The ubiquitin system degrades, in a nondiscriminatory manner, both intracellular "self" as well as foreign "non-self" proteins such as viral proteins. Peptides from both populations are presented to CTLs, but those that are derived from self proteins do not elicit a T-cell response. It is easy to imagine that aberrations in processing of these proteins may lead to presentation of differently cleaved self pep-

tides that will be recognized now as non-self. Presentation of self antigen as non-self can potentially underlie the pathogenesis of autoimmune diseases.

A wide array of immune and inflammatory disorders can also be caused by untoward activation of the immune system central transcription factor NF-κB. Activation of the factor stimulates transcription of many cytokines, adhesion molecules, inflammatory response and stress proteins, and immune system receptors. The factor is activated by the ubiquitin system via a two-step proteolytic mechanism: *1*) limited processing of the precursor protein p105 to yield the active subunit p50 and *2*) signal-induced phosphorylation and subsequent degradation of the inhibitor IκBα that enables translocation of the factor into the nucleus where it initiates specific transcriptional activity (222).

As described above, the HPV evolved a mechanism for proteolytic removal of p53 that enables continuous replication and propagation of the virus under conditions of DNA damage that normally would have ended with p53-induced apoptosis. Three other viruses evolved mechanisms that also utilize the ubiquitin system, here to escape immune surveillance. In one case, the EBV nuclear antigen 1 (EBNA1) persists in healthy virus carriers for life and is the only viral protein regularly detected in all EBV-associated malignancies, such as Burkitt's lymphoma. Unlike EBNAs 2–4 that are strong immunogens, EBNA1 is not processed and cannot elicit a CTL response. The persistence of EBNA1 contributes, most probably, to some of the pathologies caused by the virus. An interesting structural feature common to all EBNA1 proteins derived from different EBV strains is a relatively long and unusual Gly-Ala repeat at the COOH-terminal domain of the molecule. Transfer of a strong antigenic epitope from EBNA4 to EBNA1 prevented its presentation, while its insertion in an EBNA1 mutant that lacks the Gly-Ala repeat results in its presentation to the appropriate CTL. Similarly, insertion of the Gly-Ala repeat to EBNA4 inhibited CTL recognition of EBNA4-derived antigenic peptides. Thus the Gly-Ala repeat constitutes a *cis*-acting element that inhibits antigen processing and subsequent presentation of potential antigenic epitopes (267). It has been shown that while EBNA4 is degraded in an ATP-, ubiquitin-, and proteasome-dependent manner, EBNA1 is resistant to proteolysis. EBNA1 will be degraded, however, by the ubiquitin/proteasome system if the Gly-Ala repeat is deleted (267). Thus the evolution of the Gly-Ala repeat enabled the virus to evade proteolysis and subsequent presentation to the immune system. An additional interesting observation involves the pathobiology of the human cytomegalovirus (CMV). The virus genome encodes two ER resident proteins, US2 and US11, that downregulate the expression of MHC class I heavy chain molecules. The MHC molecules are synthesized and transported to the ER where they are glycosylated, but shortly thereafter, in cells expressing US2 or US11, they are transported back to the cytosol, deglycosylated, and degraded by the proteasome after ubiquitination (405). It appears that the viral products bind to the MHC molecules and escort them to the translocation machinery where they are transported back into the cytosol. The detailed mechanism of action of the viral proteins is not known. They may diffuse laterally in the membrane and interact with the emerging nascent MHC chain, an interaction that does not allow insertion of the stop-transfer signal and proper anchoring of the molecule in the membrane. Alternatively, they may compete with binding of the ER chaperone BIP that may be necessary for proper folding of the MHC molecule. In any event, the removal of the MHC molecules does not allow presentation of the viral antigenic peptides, thus enabling the virus to evade the immune system. In a similar mechanism, the Herpes virus encodes a novel family of ubiquitin ligases, E3s, the prototype of which contains a PHD domain, that also target MHC class I molecules for degradation (66a). Thus removal of the antigen carrier via different ubiquitin system-related mechanisms has become a common mechanistic theme for viruses to evade immune surveillance.

Thus it appears that evolution of many viruses involved intimate recognition with a variety of proteolytic processes. This enabled the evolution of viral mechanisms that enhance the function, via subversion of the normal proteolytic machinery, of the viral replication and propagation machinery.

G. Muscle Wasting

The ubiquitin system plays major roles in pathophysiological processes in the muscle. Muscle degeneration that follows long-term immobilization, denervation, and severe catabolic states such as occurs in sepsis and cancer-induced cachexia leads to activation of the ubiquitin pathway and induction of many of its enzymatic components. This in turn results in massive degradation of muscle proteins (260, 308). Interestingly, it appears that N-end rule pathway plays a major role in stress-induced muscle proteolysis. It is not clear how and via what mechanisms muscle proteins are converted into N-end rule pathway substrates, and its role in the process still requires further substantiation. Also, the nature of signaling mechanisms is still obscure, and it appears that cytokines such as tumor necrosis factor-α and interleukin-6 are not involved, at least not directly. Several ubiquitin ligases induced during muscle atrophy have been described recently (37a, 130b); however, their role, mode of regulation, and target substrates have remained unknown. Furthermore, none appears to be similar to the N-end rule ligase E3α.

H. Diseases Associated With Animal Models

Three interesting pathological states have been described in animal models that may have also implications for human diseases. Inactivation of HR6B, one of the mammalian homologs of Ubc2/Rad6, the E2 involved in DNA repair and in targeting "N-end rule" pathway and other protein substrates, leads to a single defect, male sterility, due to defects in spermatogenesis. The target substrate proteins may be histones, since their degradation is critical for postmeiotic chromatin remodeling that occurs during spermatogenesis (371). Another interesting case is that of the *Itch* locus in mice that encodes for a novel E3 enzyme. Defects in the locus resulted in a variety of syndromes that affect the immune system. Some animals develop an inflammatory disease of the large intestine. Others develop on a fatal disease characterized by pulmonary interstitial inflammation and alveolar proteinosis, inflammation of the stomach and skin glands that results in severe and constant itching and scarring, and hyperplasia of the lymphoid and hematopoietic cells (341). Itch was reported recently to target the intracellular domain of the transcription factor Notch (358). However, it is not clear whether accumulation of Notch, which may be due to inactivation of Itch, leads to the Itchy syndrome, or whether it is a different target protein that accumulates and leads to the observed phenotype. In a different case, mice were developed that lack *Ntan1*, the gene that codes for asparagine-specific NH_2-terminal amidase. The enzyme hydrolyzes the amide group of NH_2-terminal asparagine and converts it to aspartate, a strong secondary N-end rule "destabilizing" residue. Following arginylation, the protein is recognized by site I of E3α and is targeted for rapid ubiquitination and degradation. The *Ntan1(−/−)* mice were fertile and outwardly normal, but differed from their congenic wild-type counterparts in spontaneous activity, spatial memory, and a socially conditioned exploratory phenotype that has not been previously described in other mouse strains (249).

I. Drug Development for Targeting Aberrant Activity of the Ubiquitin System

Because of the central role the ubiquitin system plays in such a broad array of basic cellular processes, development of drugs that modulate the activity of the system may be difficult. Inhibition of enzymes common to the entire pathway, such as the proteasome, may affect many processes nonspecifically, although a narrow window between beneficial effects and toxicity can be identified for a short-term treatment. Recent experimental evidence strongly suggests that such inhibitors may indeed be beneficial in certain pathologies, such as in cancer (130), asthma (94), brain infarct (348), and autoimmune encephalomyelitis (450). In malignancies, the drugs may act via inhibition of degradation of different cell cycle inhibitors, whereas in neuroprotection they may act via inhibiting activation of NF-κB, which elicits an inflammatory response. In autoimmune diseases, they may act by inhibiting presentation of self peptides, but also by interfering with signal transduction along cellular immune cascades. A completely different approach to drug development can be, however, the development of small molecules that bind and inhibit specific E3s. For example, specific phosphopeptide derivatives that span the phosphorylation targeting domains in different substrates can serve as "baits" to the respective E3s (486, 487). This approach can turn, however, into a double-edge sword. In the case of p27 and IκBα, where phosphorylation destabilizes negative regulators, inhibition of the E3 can control disregulated cell cycle and decrease untoward activity of the immune system. Thus compounds that exert such activity can be thought of as potential drugs for the treatment of certain forms of malignancies and uncontrolled inflammatory states, respectively. However, the similarity between the phosphorylation sites of IκBα and β-catenin may lead also to stabilization of β-catenin, which is an activator, and its excessive transcriptional activity can result in malignant transformation of benign cells. A better approach may be the development of small molecules that are substrate specific and bind, preferably, to specific substrates or to their ancillary proteins rather than to an E3. When accelerated degradation of a tumor suppressor results in exposure of cells to malignant transformation, selective inhibition of the recognition machinery can potentially reverse the malignant phenotype. Peptides that bind specifically to HPV-E6 and prevent its association with p53 can interfere with p53 targeting. They were able to induce p53 in HPV-transformed cells with subsequent reversal of certain malignant characteristics or induction of apoptosis (50). Treatment directed at increasing the level of $p27^{Kip1}$ resulted in regression of the malignant phenotype in experimental models. Although it is not clear that they act via the ubiquitin system, interleukin-6 (312) and phenylacetate (326), for example, lead to G_1 arrest by increasing the level of p27.

XI. CONCLUDING REMARKS

Evidently, no part of the cell is out of reach of the ubiquitin-proteasome regulatory system. Levels of proteins in the nucleus, cytoplasm, ER lumen, as well as membrane proteins, are all kept in check by the ubiquitinating enzymes and the proteasome. Even the stability of mRNA can be regulated via proteasome-dependent protein degradation (258). As an additional outcome of this system, the peptide products of the proteasome are a critical factor in deciding whether a cell will be recog-

nized as infected or tumorigenic by the immune system and destroyed, or recognized as self and spared.

One can easily conclude that the versatility of protein ubiquitination as a cellular regulatory mechanism is now well established and appears to be comparable to that of phosphorylation, another well-studied protein modification. Indeed, as we have seen in many cases, protein phosphorylation and ubiquitination go hand in hand in the regulation of many cellular processes when phosphorylation typically precedes ubiquitination. However, the enzymology of ubiquitination appears to be more complex than that of protein phosphorylation, and the mechanism of polyubiquitin chain formation is currently not clear. For example, we do not have answers to basic questions such as whether the E3 enzymes conjugate intact chains to the substrate or whether they synthesize the chain de novo on the substrate. In the latter case, how does the enzyme reach the distal end of the growing chain? How intimately are the ubiquitination and the proteasome machineries linked? And ultimately, how are polyubiquitin-tagged proteins recognized by the proteasome for degradation?

What we can certainly expect in the near future is the identification of an ever-increasing number of substrates of the ubiquitin system and their specific E2/E3 complexes. It remains to be seen whether all the new E3s will belong to the known classes or whether new types of E3s will be identified. Consequently, but also in parallel, we can expect to see the development of an exciting area, that of specific modulators/drugs that can interfere with specific substrate recognition at different levels of the system in a variety of pathological states.

We thank an anonymous reviewer who meticulously went over the manuscript and provided us with extremely constructive comments.

Research in the laboratory of A. Ciechanover is supported by grants from the Israel Science Foundation founded by the Israeli Academy of Sciences and Humanities-Centers of Excellence Program, the German-Israeli Foundation for Scientific Research and Development, the United States-Israel Binational Science Foundation, the German-Israeli Project Cooperation, a TMR grant from the European Community, CaP Cure Foundation, the Foundation for Promotion of Research at the Technion, and a research grant administered by the Vice President of the Technion for Research. Research in the laboratory of M. Glickman is supported by grants from the Israeli Academy of Sciences and Humanities, the United States-Israel Binational Science Foundation, the Israeli Ministry of Science, the Israel Cancer Research Foundation, the Foundation for Promotion of Research at the Technion, and a research grant administered by the Vice President of the Technion for Research.

Address for reprint requests and other correspondence: M. Glickman, Faculty of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel (E-mail: glickman@tx.technion.ac.il).

REFERENCES

1. Abriel H, Loffing J, Rebhun JF, Pratt JH, Schild L, Horisberger JD, Rotin D, and Staub O. Defective regulation of the epithelial Na^+ channel by Nedd4 in Liddle's syndrome. *J Clin Invest* 103: 667–673, 1999.
2. Abu Hatoum O, Gross-Mesilaty S, Breitschopf K, Hoffman A, Gonen H, Ciechanover A, and Bengal E. Degradation of myogenic transcription factor MyoD by the ubiquitin pathway in vivo and in vitro: regulation by specific DNA binding. *Mol Cell Biol* 18: 5670–5677, 1998.
3. Abu-Shaar M and Mann RS. Generation of multiple antagonistic domains along the proximodistal axis during *Drosophila* leg development. *Development* 125: 3821–3830, 1998.
4. Adams GM, Crotchett B, Slaughter CA, DeMartino GN, and Gogol EP. Formation of proteasome-PA700 complexes directly correlates with activation of peptidase activity. *Biochemistry* 37: 12927–12932, 1998.
5. Adams GM, Falke S, Goldberg AL, Slaughter CA, DeMartino GN, and Gogol EP. Structural and functional effects of PA700 and modulator protein on proteasomes. *J Mol Biol* 273: 646–657, 1997.
6. Alberts B and Miake-Lye R. Unscrambling the puzzle of biological machines: the importance of details. *Cell* 68: 415–420, 1992.
7. Albrecht U, Sutcliffe JS, Cattanach BM, Beechey CV, Armstrong D, Eichele G, and Beaudet AL. Imprinted expression of the murine Angelman syndrome gene, Ube3a, in hippocampal and Purkinje neurons. *Nat Genet* 17: 75–78, 1997.
8. Alves-Rodrigues A, Gregori L, and Figueiredo-Pereira ME. Ubiquitin, cellular inclusions, and their role in neurodegeneration. *Trends Neurosci* 21: 516–520, 1998.
9. Amerik AY and Hochstrasser M. Analysis of the deubiquitinating enzymes of the yeast *S. cerevisiae*. *Biol Chem* 9–10: 981–992, 2000.
10. Amerik AY, Swaminathan S, Krantz BA, Wilkinson KD, and Hochstrasser M. In vivo disassembly of free polyubiquitin chains by yeast Ubp14 modulates rates of protein degradation by the proteasome. *EMBO J* 16: 4826–4838, 1997.
11. Amos LA and Cross RA. Structure and dynamics of molecular motors. *Curr Opin Struct Biol* 7: 239–246, 1997.
12. Aravind L and Koonin EV. The U box is a modified RING finger: a common domain in ubiquitination. *Curr Biol* 10: R132–R134, 2000.
13. Aravind L and Ponting CP. Homologues of 26S proteasome subunits are regulators of transcription and translation. *Protein Sci* 7: 1250–1254, 1998.
14. Aristarkhov A, Eytan E, Moghe A, Admon A, Hershko A, and Ruderman JV. E2-C, a cyclin-selective ubiquitin-carrier protein required for the destruction of mitotic cyclins. *Proc Natl Acad Sci USA* 93: 4294–4298, 1996.
15. Armon T, Ganoth D, and Hershko A. Assembly of the 26S complex that degrades proteins ligated to ubiquitin is accompanied by the formation of ATPase activity. *J Biol Chem* 265: 20723–20726, 1990.
16. Arnason T and Ellison MJ. Stress resistance in *Saccharomyces cerevisiae* is strongly correlated with assembly of a novel type of multiubiquitin chain. *Mol Cell Biol* 14: 7876–7883, 1994.
17. Asano K, Kinzy TG, Merrick WC, and Hershey JWB. Conservation and diversity of eukaryotic translation initiation factor 3. *J Biol Chem* 272: 1101–1109, 1997.
18. Aviel S, Winberg G, Masucci MG, and Ciechanover A. Degradation of the Epstein-Barr virus latent membrane protein 1 (LMP1) by the ubiquitin-proteasome pathway. Targeting via ubiquitination of the NH_2-terminal residue. *J Biol Chem* 275: 23491–23499, 2000.
19. Baboshina OV and Haas AL. Novel multiubiquitin chain linkages catalyzed by the conjugating enzymes E2-EPF and RAD6 are recognized by the 26S proteasome subunit 5. *J Biol Chem* 271: 2823–2831, 1996.
20. Bachmair AD, Finley DJ, and Varshavsky A. In vivo half-life of a protein is a function of its amino-terminal residue. *Science* 234: 179–186, 1986.
21. Bai C, Sen P, Hoffman K, Ma L, Goebl M, Harper JW, and Elledge SJ. SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* 86: 263–274, 1997.
22. Bauer MW, Bauer SH, and Kelly RM. Purification and characterization of a proteasome from the hyperthermophilic archaeon *Pyrococcus furiosus*. *Appl Environ Microbiol* 63: 1160–1164, 1997.

23. Bays NW, Gardner RG, Seelig LP, Joazeiro CAP, and Hampton RY. Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation. *Nature Cell Biol* 3: 24–29, 2001.

23a. Bays NW, Wilhovshy SK, Goradia A, Hodgkiss-Harlow K, and Hampton RY. HRD4/NPL4 is required for the proteasomal processing of ubiquitinated ER proteins. *Mol Biol Cell* 12: 4114–4128, 2001.

24. Bech-Otschir D, Kraft R, Huang X, Henklein P, Kapelari B, Pollmann C, and Dubiel W. COP9 signalosome-specific phosphorylation targets p53 to degradation by the ubiquitin system. *EMBO J* 20: 1630–1639, 2001.

25. Bence NF, Sampat RM, and Kopito RR. Impairment of the ubiquitin system by protein aggregation. *Science* 292: 1552–1555, 2001.

26. Beninga J, Rock KL, and Goldberg AL. Interferon-gamma can stimulate post-proteasomal trimming of the N terminus of an antigenic peptide by inducing leucine aminopeptidase. *J Biol Chem* 273: 18734–18742, 1998.

27. Bennett MC, Bishop JF, Leng Y, Chock PB, Chase TN, and Mouradian MM. Degradation of a-synuclein by proteasome. *J Biol Chem* 274: 33855–33858, 1999.

28. Ben-Shahar S, Komolsh A, Nadav E, Shaked I, Ziv T, Admon A, DeMartino GN, and Reiss Y. 26S proteasome-mediated production of an authentic MHC class I-restricted epitope from an intact protein substrate. *J Biol Chem* 274: 21963–21972, 1999.

29. Bercovich B, Stancovski I, Mayer A, Blumenfeld N, Laszlo A, Schwatz AL, and Ciechanover A. Ubiquitin-dependent degradation of certain protein substrates in vitro requires the molecular chaperone Hsc70. *J Biol Chem* 272: 9002–9010, 1997.

30. Berezutskaya E and Bagchi S. The human papillomavirus E7 oncoprotein functionally interacts with the S4 subunit of the 26S proteasome. *J Biol Chem* 272: 30135–30140, 1997.

31. Berleth ES and Pickart CM. Mechanism of ubiquitin conjugating enzyme E2–230K: catalysis involving a thiol relay? *Biochemistry* 35: 1664–1671, 1966.

32. Bertolaet BL, Clarke DJ, Wolff M, Watson MH, Henze M, Divita G, and Reed SI. UBA domains of DNA damage-inducible proteins interact with ubiquitin. *Nat Struct Biol* 8: 417–422, 2001.

33. Beyer A. Sequence analysis of the AAA protein family. *Protein Sci* 6: 2043–2058, 1997.

34. Biederer T, Volkwein C, and Sommer T. Degradation of subunits of the Sec61p complex, an integral component of the ER membrane, by the ubiquitin-proteasome pathway. *EMBO J* 15: 2069–2076, 1996.

35. Biederer T, Volkwein C, and Sommer T. Role of Cue1p in ubiquitination and degradation at the ER surface. *Science* 278: 1806–1809, 1997.

36. Blondel M, Galan M, Chi Y, Lafourcade C, Longaretti C, Deshaies RJ, and Peter M. Nuclear-specific degradation of Far1 is controlled by localization of the F-box protein Cdc4. *EMBO J* 19: 6085–6097, 2000.

37. Bochtler M, Ditzel M, Groll M, Hartmann C, and Huber R. The proteasome. *Annu Rev Biophys Biomol Struct* 28: 295–317, 1999.

37a. Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD, and Glass DJ. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294: 1704-1708, 2001

38. Bonifacino JS and Weissman AM. Ubiquitin and the control of protein fate in the secretory and endocytic pathways. *Annu Rev Cell Dev Biol* 14: 19–57, 1998.

39. Borden KL. RING domains: master builders of molecular scaffolds? *J Mol Biol* 295: 1103–1112, 2000.

39a. Borodovsky A, Kessler BM, Casagrande R, Overkleeft HS, Wilkinson KD, and Ploegh HL. A novel active site-directed probe specific for deubiquitylating enzymes reveals proteasome association of USP14. *EMBO J* 20: 5187–5196, 2001.

40. Bounpheng MA, Dimas JJ, Dodds SG, and Christy BA. Degradation of Id proteins by the ubiquitin-proteasome pathway. *FASEB J* 13: 2257–2264, 1999.

41. Bousiotis VA, Freedman GJ, Taylor PA, Berezovskaya A, Grass I, Blazar RB, and Nadler LM. $p27^{kip1}$ functions as an anergy factor inhibiting interleukin2 transcription and clonal expansion of alloreactive human and mouse T lymphocytes. *Nat Med* 6: 290–297, 2000.

42. Boyd SD, Tsai KY, and Jacks T. An intact HDM2 RING-finger domain is required for nuclear exclusion of p53. *Nature Cell Biol* 2: 563–568, 2000.

43. Boyer SN, Wazer DE, and Band V. E7 protein of human papilloma virus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. *Cancer Res* 56: 4620–4624, 1996.

44. Braun BC, Glickman MH, Kraft R, Dahlmann B, Kloetzel PM, Finely D, and Schmidt M. The base of the proteasome regulatory particle exhibits chaperone-like activity. *Nature Cell Biol* 1: 221–226, 1999.

45. Breitschopf K, Bengal E, Ziv T, Admon A, and Ciechanover A. A novel site for ubiquitination: the N-terminal residue, and not internal lysines of MyoD, is essential for conjugation and degradation of the protein. *EMBO J* 17: 5964–5973, 1998.

46. Brodsky JL and McCracken AA. ER protein quality control and proteasome-mediated protein degradation. *Semin Cell Dev Biol* 10: 507–513, 1999.

47. Brooks P, Fuertes G, Murray RZ, Bose S, Knecht E, Rechsteiner MC, Hendil KB, Tanaka K, Dyson J, and Rivett J. Subcellular localization of proteasomes and their regulatory complexes in mammalian cells. *Biochem J* 346: 155–161, 2000.

47a. Bruick RK and McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* 294: 1337–1340, 2001.

48. Brusky J, Zhu Y, and Xiao W. UBC13, a DNA-damage-inducible gene, is a member of the error-free postreplication repair pathway in *Saccharomyces cerevisiae*. *Curr Genet* 37: 168–174, 2000.

49. Buschmann T, Fuchs SY, Lee CG, Pan ZQ, and Ronai Z. SUMO-1 modification of Mdm2 prevents its self-ubiquitination and increases Mdm2 ability to ubiquitinate p53. *Cell* 101: 753–762, 2000.

50. Butz K, Denk C, Ullmann A, Scheffner M, and Hoppe-Seyler F. Induction of apoptosis in human papillomavirus positive cancer cells by peptide aptamers targeting the viral E6 oncoprotein. *Proc Natl Acad Sci USA* 97: 6693–6697, 2000.

51. Cadavid AL, Ginzel A, and Fischer JA. The function of the *Drosophila* fat facets deubiquitinating enzyme in limiting photoreceptor cell number is intimately associated with endocytosis. *Development* 127: 1727–1736, 2000.

51a. Cagney G, Uetz P, and Fields S. Two-hybrid analysis of the *Saccharomyces cerevisiae* 26S proteasome. *Physiol Genomics* 7: 27–34, 2001.

52. Carrano AC, Eytan E, Hershko A, and Pagano M. Skp2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nature Cell Biol* 1: 193–199, 1999.

53. Cerundolo V, Benham A, Braud V, Mukherjee S, Gould K, Macino B, Neefjes J, and Townsend A. The proteasome-specific inhibitor lactacystin blocks presentation of cytotoxic T lymphocyte epitopes in human and murine cells. *Eur J Immunol* 7: 336–341, 1997.

54. Chamovitz DA and Segal D. JAB1/CSN5 and the COP9 signalosome. A complex situation. *EMBO Reports* 2: 96–101, 2001.

55. Chamovitz DA, Wei N, Osterlund MT, von Arnim AG, Staub JM, and Deng XW. The COP9 complex, a novel multisubunit nuclear regulator involved in light control of a plant development switch. *Cell* 86: 115–121, 1996.

56. Chauchereau A, Georgiakaki M, Perrin-Wolff M, Milgrom E, and Loosfelt H. JAB1 interacts with both the progesterone receptor and SRC-1. *J Biol Chem* 275: 8540–8548, 2000.

56a. Chen L, Shinde U, Ortolan TG, and Madura K. Ubiquitin-associated (UBA) domains in Rad23 bind ubiquitin and promote inhibition of multi-ubiquitin chain assembly. *EMBO Rep* 2: 933–938, 2001.

57. Chen P and Hochstrasser M. Autocatalytic subunit processing couples active site formation in the 20S proteasome to completion of assembly. *Cell* 86: 961–972, 1996.

58. Chen P, Johnson P, Sommer T, Jentsch S, and Hochstrasser M. Multiple ubiquitin-conjugating enzymes participate in the in vivo degradation of the yeast MATa2 repressor. *Cell* 74: 357–369, 1993.

59. Chen Y, Sharp ZD, and Lee WH. HEC binds to the seventh regulatory subunit of the 26S proteasome and modulates the proteolysis of mitotic cyclins. *J Biol Chem* 272: 24081–24087, 1997.

60. Chuang L-C and Yew PR. Regulation of nuclear transport and degradation of the *Xenopus* cyclin-dependent kinase inhibitor $p27^{Xic1}$. *J Biol Chem* 276: 1610–1617, 2001.

61. Chung CH and Baek SH. Deubiquitinating enzymes: their diversity

and emerging roles. *Biochem Biophys Res Commun* 266: 633–640, 1999.
62. CIECHANOVER A, FINLEY D, AND VARSHAVSKY A. Ubiquitin dependence of selective protein degradation demonstrated in the mammalian cell cycle mutant ts85. *Cell* 37: 57–66, 1984.
63. CLARET FX, HIBI M, DHUT S, TODA T, AND KARIN M. A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. *Nature* 383: 453–457, 1996.
64. COHEN H, AZRIEL A, COHEN T, MERARO D, HASHMUELI S, BECH-OTSCHIR D, KRAFT R, DUBIEL W, AND LEVI BZ. Interaction between ICSBP and CSN2 (Trip15): a possible link between IRF signaling and the COP9/Signalosome. *J Biol Chem* 275: 39081–39089, 2000.
65. COHEN-FIX O, PETERS JM, KIRSCHNER MW, AND KOSHLAND D. Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes Dev* 10: 3081–3093, 1996.
66. CONNELL C, BALLINGER CA, JIANG J, WU Y, THOMPSON LJ, HOEHNFELD J, AND PATTERSON C. The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nature Cell Biol* 3: 93–96, 2001.
66a. COSCOY L, SANCHEZ DJ, AND GANEM D. A novel class of herpes-virus encoded membrane-bound E3 ubiquitin ligases regulates endocytosis of proteins involved in immune recognition. *J Cell Biol* 155: 1265–1273, 2001.
67. COUX O AND GOLDBERG AL. Enzymes catalyzing ubiquitination and proteolytic processing of the p105 precursor of nuclear factor NF-kB1. *J Biol Chem* 273: 8820–8828, 1998.
68. CUMMINGS CJ, REINSTEIN E, SUN Y, ANTALFFY B, JIANG Y, CIECHANOVER A, ORR HT, BEAUDET AL, AND ZOGHBI HY. Mutation of the E6-AP ubiquitin ligase reduces nuclear inclusion frequency while accelerating polyglutamine-induced pathology in SCA mice. *Neuron* 4: 879–892, 1999.
69. DAHLMANN B, KOPP F, KUEHN L, NIEDEL B, PFEIFER G, HEGERL R, AND BAUMEISTER W. The multicatalytic proteinase (prosome) is ubiquitous from eukaryotes to archaebacteria. *FEBS Lett* 251: 125–131, 1989.
69a. DAI RM AND LI C-CH. VCP is a multiubiquitin chain-targeting factor required in ubiquitin-proteasome degradation. *Nat Cell Biol* 3: 740–744, 2001.
70. D'ANDREA A AND PELLMAN D. Deubiquitinating enzymes: a new class of biological regulators. *Crit Rev Biochem Mol Biol* 33: 337–332, 1998.
71. DANTUMA NP, HEESSEN S, LINSTEN K, JELLNE M, AND MASUCCI MG. Inhibition of proteasomal degradation by the Gly-Ala repeat of Epstein-Barr virus is influenced by the length of the repeat and the strength of the degradation signal. *Proc Natl Acad Sci USA* 97: 8381–8385, 2000.
72. DAVIS NG, HORECKA JL, AND SPRAGUE GF. *Cis-* and *trans*-acting functions required for endocytosis of the yeast pheromone receptors. *J Cell Biol* 122: 53–65, 1993.
72a. DAVY A, BELLO P, THIERRY-MIEG N, VAGILO P, HITTI J, DOUCETTE-STAMM L, THIERRY-LEG, D, REBOUL J, BOULTON S, WALHOUT AJM, COUX O, AND VIDAL M. A protein-protein map of the *C. elegans* 26S proteasome. *EMBO Rep* 2: 821–828, 2001.
73. DAVYDOV IV AND VARSHAVSKY A. RGS4 is argynilated and degraded by the N-end rule pathway. *J Biol Chem* 275: 22931–22941, 2000.
74. DAWSON S, HASTINGS R, TAKAYANAGI K, REYNOLDS S, LOW P, BILLET M, AND MAYER RJ. The 26S proteasome: regulation and substrate recognition. *Mol Biol Rep* 24: 39–44, 1997.
75. DAWSON SP, ARNOLD JE, MAYER NJ, REYNOLDS SE, BILLETT MA, GORDON C, COLLEAUX L, KLOETZEL PM, TANAKA K, AND MAYER RJ. Developmental changes of the 26S proteasome in abdominal intersegmental muscles of *Manduca sexta* during programmed cell death. *J Biol Chem* 270: 1850–1858, 1995.
75a. DEBONNEVILLE C, FLORES SY, KAMYNINA E, PLANT PJ, TAUXE C, THOMAS MA, MUNSTER C, CHRAIBI A, PRATT JH, HORISBERGER JD, PEARCE D, LOFFING J, AND STAUB O. Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na^+ channel cell surface expression. *EMBO J* 20: 7052–7059, 2001.
75b. DEMAND J, ALBERTI S, PATTERSON C, AND HOHFELD J. Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. *Curr Biol* 11: 1569–1577, 2001.
76. DEMARTINO GN, MOOMAW CR, ZAGNITKO OP, PROSKE RJ, MA CP, AFENDIS SJ, SWAFFIELD JC, AND SLAUGHTER CA. PA700, an ATP-dependent activator of the 20S proteasome, is an ATPase containing multiple members of a nucleotide binding protein family. *J Biol Chem* 269: 20878–20884, 1994.
77. DEMARTINO GN, PROSKE RJ, MOOMAW CR, STRONG AA, SONG X, HISAMATSU H, TANAKA K, AND SLAUGHTER CA. Identification, purification, and characterization of a PA700-dependent activator of the proteasome. *J Biol Chem* 271: 3112–3118, 1996.
78. DE MOT R, NAGY I, WALZ J, AND BAUMEISTER W. Proteasomes and other self-compartmentalizing proteases in prokaryotes. *Trends Microbiol* 7: 88–92, 1999.
79. DENG L, WANG C, SPENCER E, LIYONG Y, BRAUN A, YOU J, SLAUGHTER C, PICKART CM, AND CHEN ZJ. Activation of the IkB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 103: 351–361, 2000.
80. DENG XW, DUBIEL W, WEI N, HOFMANN K, MUNDT K, COLICELLI J, KATO JY, NAUMANN M, SEGAL D, SEEGER M, CARR A, GLICKMAN MH, AND CHAMOVITZ DA. Unified nomenclature for the COP9 signalosome: an essential regulator of development. *Trends Genet Sci* 16: 202–203, 2000.
81. DE SEPULVEDA P, ILANGUMARAN S, AND ROTTAPEL R. Suppressor of cytokine signaling-1 inhibits VAV function through protein degradation. *J Biol Chem* 275: 14005–14008, 2000.
82. DESHAIES RJ. SCF and Cullin/RING H2-based ubiquitin ligases. *Annu Rev Cell Dev Biol* 15: 435–467, 1999.
83. DESTERRO JM, RODRIGUEZ MS, AND HAY RT. SUMO-1 modification of IkBa inhibits NF-kB activation. *Mol Cell* 2: 233–239, 1998.
84. DEVERAUX Q, JENSEN C, AND RECHSTEINER M. Molecular cloning and expression of a 26S proteasome subunit enriched in dileucine repeats. *J Biol Chem* 270: 23726–23729, 1995.
85. DEVERAUX Q, USTRELL V, PICKART C, AND RECHSTEINER M. A 26S subunit that binds ubiquitin conjugates. *J Biol Chem* 269: 7059–7061, 1994.
86. DEVERAUX Q, VAN NOCKER S, MAHAFFEY D, VIERSTRA R, AND RECHSTEINER M. Inhibition of ubiquitin-mediated proteolysis by the *Arabidopsis* 26S protease subunit s5a. *J Biol Chem* 270: 29660–29663, 1995.
87. DICK LR, ALDRICH C, JAMESON SC, MOOMAW CR, PRAMANIK BC, DOYLE CK, DEMARTINO GN, BEVAN MJ, FORMAN JM, AND SLAUGHTER CA. Proteolytic processing of ovalbumin and beta-galactosidase by the proteasome to a yield antigenic peptides. *J Immunol* 152: 3884–3894, 1994.
88. DICK TP, NUSSBAUM AK, DEEG M, HEINEMEYER W, GROLL M, SCHIRLE M, KEILHOLZ W, STEFANOVIC S, WOLF DH, HUBER R, RAMMENESEE HG, AND SCHILD H. Contribution of proteasomal beta-subunits to the cleavage of peptide substrates analyzed with yeast mutants. *J Biol Chem* 273: 25637–25646, 1998.
89. DIMMELER S, BREITSCHOPF K, HAENDELER J, AND ZEIHER AM. Dephosphorylation targets Bcl-2 for ubiquitin-dependent degradation: a link between the apoptosome and the proteasome pathway. *J Exp Med* 189: 1815–1822, 1999.
90. DOBSON CM AND ELLIS RJ. Protein folding and misfolding inside and outside the cell. *EMBO J* 17: 5251–5254, 1998.
91. DUBIEL W, FERRELL K, AND RECHSTEINER M. Subunits of the regulatory complex of the 26S proteasome. *Mol Biol Rep* 21: 27–34, 1995.
92. DUBIEL W, PRATT G, FERRELL K, AND RECHSTEINER M. Purification of an 11S regulator of the multicatalytic protease. *J Biol Chem* 267: 22369–22377, 1992.
93. DUNN R AND HICKE L. Multiple roles for Rsp5p-dependent ubiquitination at the internalization step of endocytosis. *J Biol Chem* 276: 25974–25981, 2001.
94. ELLIOTT PJ, PIEN CS, MCCORMACK TA, CHAPMAN ID, AND ADAMS J. Proteasome inhibition: a novel mechanism to combat asthma. *J Allergy Clin Immunol* 104: 294–300, 1999.
95. EMMERICH NPN, NUSSBAUM AK, STEVANOVIC S, PRIEMER M, TOES RE, RAMMENSEE HG, AND SCHILD H. The human 26S and 20S proteasomes generate overlapping but different sets of peptide fragments from a model protein substrates. *J Biol Chem* 275: 21140–21148, 2000.
96. EMSLEY J, CRUZ M, HANDIN R, AND LIDDINGTON R. Crystal structure of the von Willebrand Factor A1 domain and implications for the binding of platelet glycoprotein Ib. *J Biol Chem* 273: 10396–10401, 1998.
97. ENENKEL C, LEHMANN A, AND KLOETZEL PM. Subcellular distribution

of proteasomes implicates a major location of protein degradation in the nuclear envelope-ER network in yeast. *EMBO J* 17: 6144–6154, 1998.

98. Eytan E, Armon T, Heller H, Beck S, and Hershko A. Ubiquitin C-terminal hydrolase activity associated with the 26S protease complex. *J Biol Chem* 268: 4668–4674, 1993.
99. Fabunmi RP, Wigley WC, Thomas PJ, and DeMartino GN. Activity and regulation of the centrosome-associated proteasome. *J Biol Chem* 275: 409–413, 2000.
100. Fang G, Yu H, and Kirschner MW. The checkpoint protein MAD2 and the mitotic regulator CDC20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation. *Genes Dev* 12: 1871–1883, 1998.
101. Fergusson J, Landon M, Lowe J, Ward L, van Leeuwen FW, and Mayer RJ. Neurofibrillary tangles in progressive supranuclear palsy brains exhibit immunoreactivity to frameshift mutant ubiquitin-B protein. *Neurosci Lett* 279: 69–72, 2000.
102. Ferrell K, Wilkinson CR, Dubiel W, and Gordon C. Regulatory subunit interactions of the 26S proteasome, a complex problem. *Trends Biochem Sci* 25: 83–88, 2000.
103. Finley D, Tanaka K, Mann C, Feldmann H, Hochstrasser M, Vierstra R, Johnston S, Hampton R, Haber J, Silver P, Frontali L, Thorsness P, Varshavsky A, Byers B, Wolf D, Jentsch S, Sommer T, Baumeister W, Goldberg A, Fried V, Rubin D, Glickman M, and Toh-e A. Unified nomenclature for subunits of the S. *cerevisiae* proteasome regulatory particle. *Trends Biochem Sci* 271: 244–245, 1998.
104. Fisk HA and Yaffe MP. A role for ubiquitination in mitochondrial inheritance in *Saccharomyces cerevisiae*. *J Cell Biol* 145: 1199–1208, 1999.
105. Floyd ZE, Trausch-Azar JS, Reinstein E, Ciechanover A, and Schwartz AL. The nuclear ubiquitin-proteasome system degrades MyoD. *J Biol Chem* 276: 22468–22475, 2001.
106. Foss GS and Prydz H. Interferon regulatory factor 1 mediates the interferon-gamma induction of the human immunoproteasome subunit MECL-1. *J Biol Chem* 274: 35196–35202, 1999.
107. Freedman DA and Levine AL. Nuclear export is required for degradation of endogenous p53 by MDM2 and human papillomavirus E6. *Mol Cell Biol* 18: 7288–7293, 1998.
108. Freilich S, Oron E, Orgad S, Segal D, and Chamovitz DA. The COP9 complex is essential for development of *Drosophila melanogaster*. *Curr Biol* 9: 1187–1190, 1999.
109. Friedlander R, Jarosch E, Urban J, Volkwein C, and Sommer T. A regulatory link between ER-associated protein degradation and the unfolded protein response. *Nature Cell Biol* 2: 379–384, 2000.
109a. Fu HY, Reis N, Lee Y, Glickman MH, and Vierstra R. Subunit interaction maps for the regulatory particle of the 26s proteasome and the cop9 signalosome reveal a conserved core structure. *EMBO J* 20: 7096–7107, 2001.
110. Fu H, Sadis S, Rubin DM, Glickman MH, van Nocker S, Finley D, and Vierstra RD. Multiubiquitin chain binding and protein degradation are mediated by distinct domains within the 26S proteasome subunit Mcb1. *J Biol Chem* 273: 1970–1989, 1998.
111. Fujimuro M, Takada H, Saeki Y, Toh-e A, Tanaka K, and Yokosawa H. Growth-dependent change of the 26S proteasome in budding yeast. *Biochem Biophys Res Commun* 251: 818–823, 1998.
112. Fujimuro M, Tanaka K, Yokosawa H, and Toh-e A. Son1p is a component of the 26S proteasome of the yeast *Sacharomyces cerevisiae*. *FEBS Lett* 423: 149–154, 1998.
113. Funabiki H, Yamano H, Kumada K, Nagao K, Hunt T, and Yanagida M. Cut2 proteolysis required for sister-chromatid separation in fission yeast. *Nature* 381: 438–441, 1996.
114. Furukawa K, Mizushima N, Noda T, and Ohsumi Y. A protein conjugation system in yeast with homology to biosynthetic enzyme reaction of prokaryotes. *J Biol Chem* 275: 7462–7465, 2000.
115. Gaczynska M, Rock KL, and Goldberg AL. Gamma-interferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. *Nature* 365: 264–267, 1993.
116. Galan J and Haguenauer-Tsapis R. Ubiquitin Lys63 is involved in ubiquitination of a yeast plasma membrane protein. *EMBO J* 16: 5847–5854, 1997.
117. Galan JM, Moreau V, Andre B, Volland C, and Haguenauer-Tsapis R. Ubiquitination mediated by the Npi1p/Rsp5p ubiquitin-protein ligase is required for endocytosis of the yeast uracil permease. *J Biol Chem* 271: 10946–10952, 1996.
118. Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, Grompe M, and D'Andrea AD. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol Cell* 7: 249–262, 2001.
119. Gerlinger UM, Hoffmann M, Wolf DH, and Hilt W. Yeast cycloheximide-resistant *crl* mutants are proteasome mutants defective in protein degradation. *Mol Biol Cell* 8: 2487–2499, 1997.
120. Geyer RK, Yu ZK, and Maki CG. The MDM2 RING-finger domain is required to promote p53 nuclear export. *Nature Cell Biol* 2: 569–573, 2000.
121. Gilchrist CA and Baker RT. Characterization of the UBP activity of the mouse/human Unp/Unph oncoprotein. *Biochim Biophys Acta* 1481: 297–309, 2000.
122. Gillette TG, Huang W, Russell SJ, Reed SH, Johnston SA, and Friedberg EC. The 19S complex of the proteasome regulates nucleotide excision repair in yeast. *Genes Dev* 15: 1528–1539, 2001.
123. Girod PA, Fu H, Zryd JP, and Vierstra RD. Multiubiquitin chain binding subunit MCB1 (RPN10) of the 26S proteasome is essential for developmental progression in *Physcomitrella patens*. *Plant Cell* 11: 1457–1471, 1999.
124. Glickman MH. Getting in and out of the proteasome. *Semin Cell Dev Biol* 11: 149–158, 2000.
125. Glickman MH and Coux O. Purification and characterization of proteasomes from *Saccharomyces cerevisiae*. In: *Current Protocols in Protein Science*. New York: Wiley, 2001, p. 21.5.1–21.5.17.
126. Glickman MH, Rubin DM, Coux O, Wefes I, Pfeifer G, Cjeka Z, Baumeister W, Fried VA, and Finley D. A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9/Signalosome and eIF3. *Cell* 94: 615–623, 1998.
127. Glickman MH, Rubin DM, Fried VA, and Finley D. The regulatory particle of the *S. cerevisiae* proteasome. *Mol Cell Biol* 18: 3149–3162, 1998.
128. Glickman MH, Rubin DM, Larsen CN, and Finley D. The regulatory particle of the yeast proteasome. In: *Proteasomes: The World of Regultory Proteolysis*, edited by Hilt W and Wolf DH. Georgetown, TX: Landes Bioscience, 2000, p. 71–90.
129. Goasduff T and Cederbaum AI. CYP2E1 degradation by in vitro reconstituted systems: role of the molecular chaperone hsp90. *Arch Biochem Biophys* 379: 321–330, 2000.
130. Golab J, Stoklosa T, Czajka A, Dabrowska A, Jakobisiak M, Zagozdzon R, Wojcik C, Marczak M, and Wilk S. Synergistic antitumor effects of a selective proteasome inhibitor and TNF in mice. *Anticancer Res* 20: 1717–1721, 2000.
130a. Golbik R, Lupas AN, Koretke KK, Baumeister W, and Peters J. The Janus face of the archaeal Cdc48/p97 homologue VAT: protein folding versus unfolding. *J Biol Chem* 380: 1049–1062, 1999.
130b. Gomes MD, Lecker SH, Jagoe RT, Navon A, and Goldberg AL. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc Natl Acad Sci USA* 98: 14440–14445, 2001.
131. Gonen H, Bercovich B, Orian A, Carrano AC, Takizawa C, Yamanaka K, Pagano M, Iwai K, and Ciechanover A. Identification of the ubiquitin-carrier proteins, E2s, involved in signal-induced conjugation and subsequent degradation of IkB. *J Biol Chem* 274: 14823–14830, 1999.
132. Gonen H, Schwartz AL, and Ciechanover A. Purification and characterization of a novel protein that is required for the degradation of *N*-acetylated proteins by the ubiquitin system. *J Biol Chem* 266: 19221–19231, 1991.
133. Gonen H, Stancovski I, Shkedy D, Hadari T, Bercovich B, Bengal E, Mesilati S, Abu-Chatoum O, Schwartz AL, and Ciechanover A. Isolation, characterization, and partial purification of a novel ubiquitin-protein ligase, E3: targeting of protein substrates via multiple and distinct recognition signals and conjugating enzymes. *J Biol Chem* 271: 302–310, 1996.
134. Gong L and Yeh ET. Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. *J Biol Chem* 274: 12036–12042, 1999.
135. Gorbea C and Rechsteiner M. The mammalian regulatory complex of the 26S proteasome. In: *Proteasomes: The World of Regulatory*

Proteolysis, edited by Wolf DH and Hilt W. Georgetown, TX: Landes Bioscience, 2000, p. 91–128.
136. Gorbea C, Taillander D, and Rechsteiner R. Mapping subunit contacts in the regulatory complex of the 26S proteasome. *J Biol Chem* 275: 2, 2000.
137. Gottesman S, Maurizi MR, and Wickner S. Regulatory subunits of energy-dependent proteases. *Cell* 91: 435–438, 1997.
138. Gottesman S, Wickner S, and Maurizi MR. Protein quality control: triage by chaperones and proteases. *Genes Dev* 11: 815–823, 1997.
139. Govers R, ten Broeke T, van Kerkhof P, Schwartz AL, and Strous GJ. Identification of a novel ubiquitin conjugation motif required for ligand-induced internalization of the growth hormone receptor. *EMBO J* 18: 28–36, 1999.
140. Gray DA, Inazawa J, Gupta K, Wong A, Ueda R, and Takahashi T. Elevated expression of Unph, a proto-oncogene at 3p21.3, in human lung tumors. *Oncogene* 10: 2179–2183, 1995.
141. Groettrup M, Soza A, Eggers M, Kuehn L, Dick TP, Schild H, Rammensee HG, Koszinowski UH, and Kloetzel PM. A role for the proteasome regulator PA28a in antigen presentation. *Nature* 381: 166–168, 1996.
142. Groll M, Bajorek M, Koehler A, Moroder L, Rubin D, Huber R, Glickman MH, and Finley D. A gated channel into the core particle of the proteasome. *Nat Struct Biol* 7: 1062–1067, 2000.
143. Groll M, Ditzel L, Loewe J, Stock D, Bochtler M, Bartunik HD, and Huber R. Structure of 20S proteasome from yeast at a 2.4 Angstrom resolution. *Nature* 386: 463–471, 1997.
144. Gstaiger M, Jordan R, Lim M, Catzavelos C, Mestan J, Slingerland J, and Krek W. Skp2 is oncogenic and overexpressed in human cancers. *Proc Natl Acad Sci USA* 98: 5043–5048, 2001.
145. Hadari T, Warms JVB, Rose IA, and Hershko A. A ubiquitin-C-terminal isopeptidase that acts on polyubiquitin chains: role in protein degradation. *J Biol Chem* 267: 719–727, 1992.
146. Hakak Y and Martin GS. Ubiquitin-dependent degradation of active Src. *Curr Biol* 9: 1039–1042, 1999.
147. Hampton RY and Bhakta H. Ubiquitin-mediated regulation of 3-hydroxy-3-methylglutaryl-CoA reductase. *Proc Natl Acad Sci USA* 94: 12944–12948, 1997.
148. Hampton RY, Gardner RG, and Rine J. Role of 26S proteasome and *HRD* genes in the degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, an integral endoplasmatic reticulum membrane protein. *Mol Biol Cell* 7: 2029–2044, 1996.
149. Handley PM, Mueckler M, Siegel NR, Ciechanover A, and Schwartz AL. Molecular cloning, sequence, and tissue distribution of the human ubiquitin-activating enzyme, E1. *Proc Natl Acad Sci USA* 88: 258–262, 1991.
150. Haracska L and Udvardy A. Mapping the ubiquitin-binding domains in the p54 regulatory complex subunit of the *Drosophila* 26S protease. *FEBS Lett* 412: 331–336, 1997.
151. Hart M, Concordet JP, Lassot I, Albert I, del los Santos R, Durand H, Perret C, Rubinfeld B, Margottin F, Benarous R, and Polakis P. The F-box protein beta-TrCP associates with phosphorylated beta-catenin and regulates its activity in the cell. *Curr Biol* 9: 207–210, 1999.
152. Hartmann-Petersen R, Tanaka K, and Hendil KB. Quaternary structure of the ATPase complex of human 26S proteasomes determined by chemical cross-linking. *Arch Biochem Biophys* 386: 89–94, 2001.
153. Hatakeyama S, Yada M, Matsumoto M, Ishida N, and Nakayama K-I. U-box proteins as a new family of ubiquitin-protein ligases. *J Biol Chem.* 276: 33111–33120, 2001.
154. Hauser HP, Bardroff M, Pyrowolakis G, and Jentsch S. A giant ubiquitin-conjugating enzyme related to IAP apoptosis inhibitors. *J Cell Biol* 141: 1415–1422, 1998.
155. Hedge AN, Inokuchi K, Pei W, Casadio A, Ghirardi M, Kandel ER, and Schwartz JH. Ubiquitin C-terminal hydrolase is an immediate early gene essential for long term facilitation in *Aplysia*. *Cell* 89: 115–126, 1997.
156. Heinemeyer W and Wolf DH. Active sites and assembly of the 20S proteasome. In: *Proteasomes: The World of Regulatory Proteolysis*, edited by Wolf DH and Hilt W. Georgetown, TX: Landes Bioscience, 2000, p. 48–70.
157. Heissmeyer V, Krappmann D, Hatada EN, and Scheidereit C. Shared pathways of IkB kinase-induced SCF-betaTrCP-mediated ubiquitination and degradation for the NF-kB precursor p105 and IkB-alpha. *Mol Cell Biol* 21: 1024–1035, 2001.
158. Heissmeyer V, Krappmann D, Wulczyn FG, and Scheidereit C. NF-kappaB p105 is a target of IkappaB kinases and controls signal induction of Bcl-3-p50 complexes. *EMBO J* 18: 4766–4778, 1999.
159. Henderson BR. Nuclear-cytoplasmic shuttling of APC regulates β-catenin subcellular localization and turnover. *Nature Cell Biol* 2: 653–660, 2000.
160. Hendil KB, Khan S, and Tanaka K. Simultaneous binding of PA28 and PA700 activators to 20S proteasomes. *Biochem J* 332: 749–754, 1998.
161. Hershko A and Ciechanover A. The ubiquitin-proteasome pathway. *Annu Rev Biochem* 67: 425–479, 1998.
162. Hershko A, Eytan E, Ciechanover A, and Haas AL. Immunochemical analysis of the turnover of ubiquitin-protein conjugates in intact cells: relationship to the breakdown of abnormal proteins. *J Biol Chem* 257: 3964–13970, 1982.
163. Hershko A, Heller H, Elias S, and Ciechanover A. Components of the ubiquitin protein ligase system: resolution, affinity purification and role in protein breakdown. *J Biol Chem* 258: 8206–8214, 1983.
164. Hershko A, Leshinsky E, Ganoth D, and Heller H. ATP-dependent degradation of ubiquitin-protein conjugates. *Proc Natl Acad Sci USA* 81: 1619–1623, 1984.
165. Heusch M, Lin L, Geleziunas R, and Greene WC. The generation of NFκB2 p52: mechanism and efficiency. *Oncogene* 18: 6201–6208, 1999.
166. Hicke L. Gettin' down with ubiquitin: turning off cell-surface receptors, transporters and channels. *Trends Cell Biol* 9: 107–112, 1999.
167. Hicke L. Protein regulation by monoubiquitin. *Nat Rev Mol Cell Biol* 2: 195–201, 2001.
168. Higashitsuji H, Itoh K, Nagao T, Dawson S, Nonoguchi K, Kido T, Mayer RJ, Arii S, and Fujita J. Reduced stability of retinoblastoma protein by gankyrin, an oncogenic ankyrin-repeat protein overexpressed in hepatomas. *Nat Med* 6: 96–99, 2000.
169. Hiller MM, Finger A, Schweiger M, and Wolf DH. ER degradation of a misfolded luminal protein by the cytosolic ubiquitin pathway. *Science* 273: 1725–1728, 1996.
170. Hilt W and Wolf DH (Editors). *Proteasomes: The World of Regulatory Proteolysis*. Georgetown, TX: Landes Bioscience, 2000.
171. Hisamoto N, Sugimoto K, and Matsumoto K. The Glc7 type 1 protein phosphatase of *Saccharomyces cerevisiae* is required for cell cycle progression in G_2/M. *Mol Cell Biol* 14: 3158–3165, 1994.
171a. Hitchcock AL, Krebber H, Frietze S, Lin A, Latterich M, and Silver PA. The conserved np14 protein complex mediates proteasome-dependent membrane-bound transcription factor activation. *Mol Biol Cell* 12: 3226–3241, 2001.
172. Hiyama H, Yokoi M, Masutani C, Sugasawa K, Maekawa T, Tanaka K, Hoeijmaker JH, and Hanaoka F. Interaction of hHR23 with s5a. The Ubl domain of hHR23 mediates interaction with s5a subuinit of 26S proteasome. *J Biol Chem* 274: 28019–28025, 1999.
173. Hochstrasser M. Evolution and function of ubiquitin-like protein-conjugating systems. *Nature Cell Biol* 2: E153–E157, 2000.
174. Hoelzl H, Kapelari B, Kellermann J, Seemuller E, Sumegi M, Udvardy A, Medalia O, Sperling J, Muller S, Engel A, and Baumeister W. The regulatory complex of *Drosophila melanogaster* 26S proteasomes. Subunit composition and localization of a deubiquitylating enzyme. *J Cell Biol* 150: 119–130, 2000.
175. Hoffman L and Rechsteiner M. Activation of the multicatalytic protease. *J Biol Chem* 269: 16890–16895, 1994.
176. Hofmann K and Bucher P. The UBA domain: a sequence motif present in multiple enzyme classes of the ubiquitination pathway. *Trends Biochem Sci* 21: 172–173, 1996.
177. Hofmann K and Bucher P. The PCI domain: a common theme in three multi-protein complexes. *Trends Biochem Sci* 23: 204–205, 1998.
178. Hofmann K and Falquet L. A ubiquitin-interacting motif conserved in components of the proteasomal and lysosomal degradation systems. *Trends Biochem Sci* 26: 347–350, 2001.
179. Hofmann RM and Pickart CM. Noncanonical MMS2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. *Cell* 96: 645–653, 1999.
180. Holzhutter HG, Froemmel C, and Kloetzel PM. A theoretical ap-

proach towards the identification of cleavage-determining amino acid motifs of the 20S proteasome. *J Mol Biol* 286: 1251–1265, 1999.
181. HOPPE T, MATUSCHEWSKI K, RAPE M, SCHLENKER S, ULRICH HD, AND JENTSCH S. Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. *Cell* 102: 577–586, 2000.
182. HORI T, KATO S, SAEKI M, DEMARTINO GN, SLAUGHTER CA, TAKEUCHI J, TOH-E A, AND TANAKA K. cDNA cloning and functional analysis of p28 (Nas6p) and p40. *5 (Nas7p)*, two novel regulatory subunits of the 26S proteasome. *Gene* 216: 113–122, 1998.
183. HORWICH AL, WEBER-BAN EU, AND FINLEY D. Chaperone rings in protein folding and degradation. *Proc Natl Acad Sci USA* 96: 11033–11040, 1999.
184. HOU D, CENCIARELLI C, JENSEN JP, NGUYGEN HB, AND WEISSMAN AM. Activation-dependent ubiquitination of a T cell antigen receptor subunit on multiple intracellular lysines. *J Biol Chem* 269: 14244–14247, 1994.
185. HOUGH R, PRATT G, AND RECHSTEINER M. Ubiquitin-lysozyme conjugates. *J Biol Chem* 261: 2400–2408, 1986.
186. HU Z, ZHANG Z, DOO E, COUX O, GOLDBERG AL, AND LIANG TJ. Hepatitis B virus X protein is both a substrate and a potential inhibitor of the proteasome complex. *J Virol* 73: 7231–7240, 1999.
187. HUANG L, KINNUCAN E, WANG G, BEAUDENON S, HOWLEY PM, HUIBREGTSE JM, AND PAVLETICH NP. Structure of an E6-AP-UbcH7 complex: insights into ubiquitination by the E2–E3 enzyme cascade. *Science* 286: 1321–1326, 1999.
188. HUANG Y, BAKER RT, AND FISCHER-VIZE JA. Control of cell fate by a deubiquitinating enzyme encoded by the fat facets gene. *Science* 270: 1828–1831, 1995.
189. HUANG Y AND FISCHER-VIZE JA. Undifferentiated cells in the developing *Drosophila* eye influence facet assembly and require the Fat facets ubiquitin-specific protease. *Development* 122: 3207–3216, 1996.
190. HUIBREGTSE JM, SCHEFFNER M, BEAUDENON S, AND HOWLEY PM. A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. *Proc Natl Acad Sci USA* 92: 2563–2567, 1995.
191. HUIBREGTSE JM, SCHEFFNER M, AND HOWLEY PM. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. *Mol Cell Biol* 13: 775–784, 1993.
192. HWANG LH, LAU LF, SMITH DL, MISTROT CA, HARDWICK KG, HWANG ES, AMON A, AND MURRAY AW. Budding yeast Cdc20: a target of the spindle checkpoint. *Science* 279: 1041–1044, 1998.
193. IRNIGER S, PIATTI S, MICHAELIS C, AND NASMYTH K. Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. *Cell* 81: 269–278, 1995.
194. ISAKSSON A, PEVERALI FA, KOCKEL L, MLODZIK M, AND BOHMANN D. The deubiquitination enzyme fat facets negatively regulates RTK/Ras/MAPK signalling during *Drosophila* eye development. *Mech Dev* 68: 59–67, 1997.
195. IVAN M, KONDO K, YANG H, KIM W, VALIANDO J, OHH M, SALIC A, ASARA JM, LANE WS, AND KAELIN WG JR. HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. *Science* 292: 464–468, 2001.
196. IWAI K, YAMANAKA K, KAMURA T, MINATO N, CONAWAY RC, CONAWAY JW, KLAUSNER RD, AND PAUSE A. Identification of the von Hippel-Lindau tumor-suppressor protein as part of an active E3 ubiquitin ligase complex. *Proc Natl Acad Sci USA* 96: 12436–12441, 1999.
197. JAAKOLA P, MOLE DR, TIAN Y-M, WILSON MJ, GIELBERT J, GASKELL SJ, VON KRIEGSHEIM A, HEBESTREIT HF, MUKHERJI M, SCHOFIELD CJ, MAXWELL PH, PUGH CW, AND RATKLIFFE PJ. Targeting of HIF-α to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science* 292: 468–472, 2001.
198. JACKSON PK, ELDRIDGE AG, FREED E, FRUSTENTHAL L, HSU JY, KAISER BK, AND REIMANN JDR. The lore of the RINGS: substrate recognition and catalysis by ubiquitin ligases. *Trends Cell Biol* 10: 429–439, 2000.
199. JAGER S, STRAYLE J, HEINEMEYER W, AND WOLF DH. Cic1, an adaptor protein specifically linking the 26S proteasome to its substrate, the SCF component Cdc4. *EMBO J* 20: 4423–4431, 2001.
200. JASPERSEN SL, CHARLES JF, AND MORGAN DO. Inhibitory phosphorylation of the APC regulator Hct1 is controlled by the kinase Cdc28 and the phosphatase Cdc14. *Curr Biol* 9: 227–236, 1999.
201. JELINSKY SA, ESTEP P, CHURCH GM, AND SAMSON LD. Regulatory networks revealed by transcriptional profiling of damaged *Saccharomyces cerevisiae* cells: Rpn4 links base excision repair with proteasomes. *Mol Cell Biol* 20: 8157–8167, 2000.
202. JENSEN DE, PROCTOR M, MARQUIS ST, GARDNER HP, HA SI, CHODOSH LA, ISHOV AM, TOMMERUP N, VISSING H, SEKIDO Y, MINNA J, BORODOVSKY A, SCHULTZ DC, WILKINSON KD, MAUL GG, BARLEV N, BERGER SL, PRENDERGAST GC, AND RAUSCHER FJ. BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. *Oncogene* 16: 1097–1112, 1998.
203. JENTSCH S, MCGRATH JP, AND VARSHAVSKY A. The yeast DNA repair gene RAD6 encodes a ubiquitin conjugating enzyme. *Nature* 329: 131–134, 1987.
204. JENTSCH S AND PYROWOLAKIS G. Ubiquitin and its kin: how close are family ties? *Trends Cell Biol* 10: 335–342, 2000.
204a. JIANG J, BALLINGER CA, WU Y, DAI Q, CYR DM, HOHFELD J, AND PATTERSON C. CHIP is a U-box-dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation. *J Biol Chem* 276: 42938–42944, 2001.
205. JIN T, GU Y, ZANUSSO G, SY MS, KUMAR A, COHEN M, GAMBETTI P, AND SINGH N. The chaperone protein BiP binds to mutant Prion protein and mediates its degradation by the proteasome. *J Biol Chem* 275: 38699–38704, 2000.
206. JOAZEIRO CA, WING SS, HUANG H, LEVERSON JD, HUNTER T, AND LIU YC. The tyrosine kinase negative regulator c-Cbl as a RING-type E2-dependent ubiquitin-protein ligase. *Science* 286: 309–312, 1999.
207. JOAZEIRO CAP AND WEISSMAN AM. RING finger proteins: mediators of ubiquitin ligase activity. *Cell* 102: 549–552, 2000.
208. JOHNSON ES AND BLOBEL G. Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. *J Biol Chem* 272: 26799–26802, 1997.
208a. JOHNSON ES AND GUPTA K. An E3-like factor that promotes SUMO conjugation to the yeast septins. *Cell* 106: 735–744, 2001.
209. JOHNSON ES, MA PC, OTA IM, AND VARSHAVSKY A. A proteolytic pathway that recognizes ubiquitin as a degradation signal. *J Biol Chem* 270: 17442–17456, 1995.
210. JOHNSON ES, SCHWIENHORST I, DOHMEN RJ, AND BLOBEL G. The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. *EMBO J* 18: 5509–5519, 1997.
211. JOHNSON PR, SWANSON R, RAKHILINA L, AND HOCHSTRASSER M. Degradation signal masking by heterodimerization of MATα2 and MATα1 blocks their mutual destruction by the ubiquitin-proteasome pathway. *Cell* 94: 217–227, 1998.
212. JOHNSTON JA, DALTON MJ, GRUNEY ME, AND KOPITO RR. Formation of high molecular weight complexes of mutant Cu-Zn superoxide dismutase in a mouse model for familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA* 97: 12571–12576, 2000.
213. JOHNSTON JA, WARD CL, AND KOPITO RR. Aggresomes: a cellular response to misfolded proteins. *J Cell Biol* 143: 1883–1898, 1998.
214. KAISER P, FLICK K, WITTENBERG C, AND REED S. Regulation of transcription by ubiquitination without proteolysis: Cdc34/SCF(Met30)-mediated inactivation of the transcription factor Met4. *Cell* 102: 303–314, 2000.
215. KAISER P, MANSOUR HA, GREETEN T, AUER B, SCHWEIGER M, AND SCHNEIDER R. The human ubiquitin-conjugating enzyme UbcH1 is involved in the repair of UV-damaged, alkylated and cross-linked DNA. *FEBS Lett* 350: 1–4, 1994.
216. KAISER P, MONCOLLIN V, CLARKE DJ, WATSON MH, BERTOLAET BL, REED SI, AND BAILLY E. Cyclin-dependent kinase and Cks/Suc1 interact with the proteasome in yeast to control proteolysis of M-phase targets. *Genes Dev* 13: 1190–1202, 1999.
217. KALCHMAN MA, GRAHAM RK, XIA G, KOIDE HB, HODGSON JG, GRAHAM KC, GOLDBERG YP, GIETZ RD, PICKART CM, AND HAYDEN MR. Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. *J Biol Chem* 271: 19385–19394, 1996.
218. KAMITANI T, KITO K, NGUYEN HP, WADA H, FUKUDA-KAMITANI T, AND YEH ET. Identification of three major sentrinization sites in PML. *J Biol Chem* 273: 26675–26682, 1998.
219. KAMURA T, KOEPP DM, CONRAD MN, SKOWYRA D, MORELAND RJ, ILIPOULOS O, LANE WS, KAELIN WG JR, ELLEDGE SJ, CONAWAY RC, HARPER JW, AND

CONAWAY JW. Rbx1, a component of the VHL tumor suppressor complex and SCF ubiquitin ligase. *Science* 284: 657–661, 1999.
220. KAMURA T, SATO S, IWAI K, CZYZYK-KRZESKA M, CONAWAY RC, AND CONAWAY JW. Activation of HIF1-α ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. *Proc Natl Acad Sci USA* 97: 10430–10435, 2000.
221. KAPELARI B, BECH-OTSCHIR D, HEGERL R, SCHADE R, DUMDEY R, AND DUBIEL W. Electron microscopy and subunit-subunit interaction studies reveal a first architecture of COP9 signalosome. *J Mol Biol* 300: 1169–1178, 2000.
222. KARIN M AND BEN-NERIAH Y. Phosphorylation meets ubiquitination: the control of NF-kB activity. *Annu Rev Immunol* 18: 621–663, 2000.
223. KARNIOL B, YAHALOM T, KWOK S, TSUGE T, MATSUI M, DENG XW, AND CHAMOVITZ DA. The *Arabidopsis* homologue of an eIF3 complex subunit associates with the COP9 complex. *FEBS Lett* 439: 173–179, 1998.
223a. KAWAKAMI T, CHIBA T, SUZUKI T, IWAI K, YAMANAKA K, MINATO N, SUZUKI H, SHIMBARA N, HIDAKA Y, OSAKA F, OMATA M, AND TANAKA K. NEDD8 recruits E2-ubiquitin to SCF E3 ligase. *EMBO J* 20: 4003–4012, 2001.
223b. KAYE FJ, MODI S, IVANOVSKA I, KOONIN EV, THRESS K, KUBO A, KORNBLUTH S, AND ROSE MD. A family of ubiquitin-like proteins binds the ATPase domain of Hsp70-like Stch. *FEBS Lett* 467: 348–355, 2000.
224. KESSEL M, MAURIZI MR, KIM B, KOCSIS E, TRUS BL, SINGH SK, AND STEVEN AC. Homology in structural organization between *E. coli* ClpAP protease and the eukaryotic 26 S proteasome. *J Mol Biol* 250: 587–594, 1995.
225. KIM TH, HOFMANN K, VON ARMIN AG, AND CHAMOVITZ DA. PCI complexes: pretty complex interactions in diverse signaling pathways. *Trends Plant Sci* 6: 379–386, 2001.
226. KIM TW, PETTINGELL WH, HALLMARK OG, MOIR RD, WASCO W, AND TANZI RE. Endoproteolytic cleavage and proteasomal degradation of Presenilin 2 in transfected cells. *J Biol Chem* 272: 11006–11010, 1997.
227. KING RW, GLOTZER M, AND KIRSCHNER MW. Mutagenic analysis of the destruction signal of mitotic cyclins and structural characterization of ubiquitinated intermediates. *Mol Biol Cell* 7: 1343–1357, 1996.
228. KING RW, PETERS JM, TUGENDREICH S, ROLFE M, HIETER P, AND KIRSCHNER MW. A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* 81: 279–288, 1995.
229. KISHINO T, LALANDE M, AND WAGSTAFF J. UBE3A/E6-AP mutations cause Angelman syndrome. *Nat Genet* 15: 70–73, 1997.
230. KISSELEV AF, AKOPIAN TN, AND GOLDBERG AL. Range of sizes of peptide products generated during degradation of different proteins by Archaeal proteasomes. *J Biol Chem* 273: 1982–1989, 1998.
231. KISSELEV AF, AKOPIAN TN, WOO KM, AND GOLDBERG AL. The size of peptides generated from protein by mammalian 26S and 20S proteasomes. *J Biol Chem* 274: 3363–3371, 1999.
232. KITADA T, ASAKAWA S, HATTORI N, MATSUMINE H, YAMAMURA Y, MINOSHIMA S, YOKOCHI M, MIZUNO Y, AND SHIMIZU N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392: 605–608, 1998.
233. KITAGAWA K, SKOWYRA D, ELLEDGE SJ, HARPER JW, AND HIETER P. SGT1 encodes an essential component of the yeast kinetochore assembly pathway and a novel subunit of the SCF ubiquitin ligase complex. *Mol Cell* 4: 21–33, 1999.
234. KITAGAWA M, HATAKEYAMA S, SHIRANE M, MATSUMOTO M, ISHIDA N, HATTORI K, NAKAMICHI I, KIKUCHI A, NAKAYAMA K, AND NAKAYAMA K. An F-box protein, FWD1, mediates ubiquitin-dependent proteolysis of β-catenin. *EMBO J* 18: 2401–2410, 1999.
235. KLEEMANN R, HAUSSER A, GEIGER G, MISCHKE R, BURGER-KENTISCHER A, FLIEGER O, JOHANNES FJ, ROGER T, CALANDRA T, KAPURNIOTU A, GRELL M, FINKELMEIER D, BRUNNER H, AND BERNHAGEN J. Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through Jab1. *Nature* 408: 211–216, 2000.
236. KLEIJNEN MF, SHIH AH, ZHOU P, KUMAR S, SOCCIO RE, KEDERSHA NL, GILL G, AND HOWLEY PM. The hPLIC proteins may provide a link between the ubiquitination machinery and the proteasome. *Mol Cell* 6: 409–419, 2000.
237. KLIONSKY DJ AND OHSUMI Y. Vacuolar import of proteins and organelles from the cytoplasm. *Annu Rev Cell Dev Biol* 15: 1–32, 1999.
238. KLOETZEL PM. Antigen processing by the proteasome. *Nat Rev Mol Cell Biol* 2: 179–187, 2001.
239. KLOTZBUCHER A, STEWART E, HARRISON D, AND HUNT T. The "destruction box" of cyclin A allows B-type cyclins to be ubiquitinated, but not efficiently destroyed. *EMBO J* 15: 3053–3064, 1996.
240. KNIPFER N AND SHRADER TE. Inactivation of the 20S poteasome in *Mycobacterium smegmatis*. *Mol Microbiol* 25: 375–383, 1997.
241. KOEGL M, HOPPE T, SCHLENKER S, ULRICH HD, MAYER TU, AND JENTSCH S. A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell* 96: 635–644, 1999.
242. KOEHLER A, CASCIO P, LEGGET DS, WOO KM, GOLDBERG AL, AND FINLEY D. The axial channel of the proteasome core particle is gated by the Rpt2 ATPase and controls both substrate entry and product release. *Mol Cell* 7: 1143–1152, 2001.
243. KOPITO RR. Biosynthesis and degradation of CFTR. *Physiol Rev* 79 *Suppl*: S167–S173, 1999.
244. KORNITZER D AND CIECHANOVER A. Modes of regulation of ubiquitin-mediated protein degradation. *J Cell Physiol* 182: 1–11, 2000.
245. KOTANI S, TUGENDREICH S, FUJII M, JORGENSEN PM, WATANABE N, HOOG C, HIETER P, AND TODOKORO K. PKA and MPF-activated polo-like kinase regulate anaphase-promoting complex activity and mitosis progression. *Mol Cell* 1: 371–380, 1998.
246. KRUGER R, MULLER T, AND RIESS OJ. Involvement of α-synuclein in Parkinson's disease and other neurodegenerative disorders. *Neural Transm* 107: 31–40, 2000.
247. KUTTLER C, NUSSBAUM AK, DICK TP, RAMMENSEE HG, SCHILD H, AND HADELER KP. An algorithm for the prediction of proteasomal cleavages. *J Mol Biol* 298: 417–429, 2000.
248. KWOK SF, STAUB JM, AND DENG XW. Characterisation of two subunits of *Arabidopsis* 19S proteasome regulatory complex and its possible interaction with the COP9 complex. *J Mol Biol* 285: 85–95, 1999.
249. KWON YT, BALOGH SA, DAVYDOV IV, KASHINA AS, YOON JK, XIE Y, GAUR A, HYDE L, DENENBERG VH, AND VARSHAVSKY A. Altered activity, social behavior, and spatial memory in mice lacking the NTAN1p amidase and the asparagine branch of the N-end rule pathway. *Mol Cell Biol* 20: 4135–4148, 2000.
250. KWON YT, REISS Y, FRIED VA, HERSHKO A, YOON JK, GONDA DK, SANGAN P, COPELAND NG, JENKINS NA, AND VARSHAVSKY A. The mouse and human genes encoding the recognition component of the N-end rule pathway. *Proc Natl Acad Sci USA* 95: 7898–7903, 1998.
251. LAAN LA, VAN HAERINGEN A, AND BROUWER OF. Angelman syndrome: a review of clinical and genetic aspects. *Clin Neurol Neurosurg* 101: 161–170, 1999.
252. LAHAV-BARATZ S, SUDAKIN V, RUDERMAN JV, AND HERSHKO A. Reversible phosphorylation controls the activity of cyclosome-associated cyclin-ubiquitin ligase. *Proc Natl Acad Sci USA* 92: 9303–9307, 1995.
253. LAI Z, FERRY KV, DIAMOND M, WEE K, KIM YB, MA J, YANG T, BENFIELD PA, COPELAND RA, AND AUGER KR. Human Mdm2 mediates multiple mono-ubiquitination of p53 by a mechanism requiring enzyme isomerization. *J Biol Chem* 276: 31357–31367, 2001.
254. LAM YA, DEMARTINO GN, PICKART CM, AND COHEN RE. Specificity of the ubiquitin isopeptidase in the PA700 regulatory complex of the 26S proteasome. *J Biol Chem* 272: 28438–28446, 1997.
255. LAM YA, PICKART CM, ALBAN A, LANDON M, JAMIESON C, RAMAGE R, MAYER RJ, AND LAYFIELD R. Inhibition of the ubiquitin-proteasome system in Alzheimer's disease. *Proc Natl Acad Sci USA* 97: 9902–9906, 2000.
256. LAM YA, XU W, DEMARTINO GN, AND COHEN RE. Editing of ubiquitin conjugates by an isopeptidase in the 26S proteasome. *Nature* 385: 737–740, 1997.
257. LAMBERTSON D, CHEN L, AND MADURA K. Pleiotropic defects caused by loss of the proteasomal-interacting factors Rad23 and Rpn10 of *S. cerevisiae*. *Genetics* 153: 69–79, 1999.
258. LAROIA G, CUESTA R, BREWER G, AND SCHNEIDER RJ. Control of mRNA decay by heat shock-ubiquitin-proteasome pathway. *Science* 284: 499–502, 1999.
259. LAYFIELD R, FRANKLIN K, LANDON M, WALKER G, WANG P, RAMAGE R, BROWN A, LOVE S, URQUHART K, MUIR T, BAKER R, AND MAYER RJ. Chemically synthesized ubiquitin extension proteins detect distinct catalytic capacities of deubiquitinating enzymes. *Anal Biochem* 274: 40–49, 1999.
260. LECKER SH, SOLOMON V, MITCH WE, AND GOLDBERG AL. Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. *J Nutr* 129: 227S–237S, 1999.
261. LEE C, SCHWARTZ MP, PRAKASH S, IWAKURA M, AND MATOUSCHEK A. ATP-

dependent proteases degrade their substrates by processively unraveling them from the degradation signal. *Mol Cell* 7: 627–637, 2001.
262. LEE DH, SHERMAN MY, AND GOLDBERG AL. Involvement of the molecular chaperone Ydj1 in the ubiquitin-dependent degradation of short-lived abnormal proteins in *Saccharomyces cerevisiae*. *Mol Cell Biol* 16: 4773–4781, 1996.
263. LEE PS, WANG Y, DOMINGUEZ MG, YEUNG YG, MURPHY MA, BOWTELL DD, AND STANLEY ER. The Cbl protooncoprotein stimulates CSF-1 receptor multiubiquitination and endocytosis and attenuates macrophage proliferation. *EMBO J* 18: 3616–3628, 1999.
264. LENK U AND SOMMER T. Ubiquitin-mediated proteolysis of a short lived regulatory protein depends on its cellular localization. *J Biol Chem* 275: 39403–3940, 2000.
265. LEROY E, BOYER R, AUBURGER G, LEUBE B, ULM G, MEZEY E, HARTA G, BROWNSTEIN MJ, JONNALAGADA S, CHERNOVA T, DEHEJIA A, LAVEDAN C, GASSER T, STEINBACH PJ, WILKINSON KD, AND POLYMEROPOULOS MH. The ubiquitin pathway in Parkinson's disease. *Nature* 395: 451–452, 1998.
266. LEVERSON JD, JOAZEIRO CAP, PAGE AM, HUANG KH, HIETER P, AND HUNTER T. The APC11 RING-H2 finger mediates E2-dependent ubiquitination. *Mol Biol Cell* 11: 2315–2325, 2000.
267. LEVITSKAYA J, CORAM M, LEVITSKY V, IMREH S, STEIGERWALD-MULLEN PM, KLEIN G, KURILLA MG, AND MASUCCI MG. Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. *Nature* 375: 685–688, 1995.
268. LEVITSKAYA J, SHARIPO A, LEONCHIKS A, CIECHANOVER A, AND MASUCCI MG. Inhibition of ubiquitin-proteasome-dependent protein degradation by the Gly-Ala repeat domain of the Epstein-Barr Virus Nuclear Antigen (EBNA)-1. *Proc Natl Acad Sci USA* 94: 12616–12621, 1997.
269. LEVKOWITZ G, WATERMAN H, ETTENBERG SA, KATZ M, TSYGANKOV AY, ALROY I, LAVI S, IWAI K, REISS Y, CIECHANOVER A, LIPKOWITZ S, AND YARDEN Y. Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol Cell* 4: 1029–1040, 1999.
270. LEVKOWITZ G, WATERMAN H, ZAMIR E, KAM Z, OVED S, LANDON WY, BEGUINOT L, GEIGER B, AND YARDEN Y. c-Cbl regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev* 12: 3663–3674, 1998.
271. LI S, LIU X, AND ASCOLI M. $p38^{JAB1}$ binds to the intracellular precursor of the lutropin/choriogonadotropin receptor and promotes its degradation. *J Biol Chem* 275: 13386–13393, 2000.
272. LI T, NAQVI NI, YANG H, AND TEO TS. Identification of a 26S proteasome-associated UCH in fission yeast. *Biochem Biophys Res Commun* 272: 270–275, 2000.
273. LIAKOPOULOS D, DOENGES G, MATUSCHEWSKI K, AND JENTSCH S. A novel protein modification pathway related to the ubiquitin system. *EMBO J* 17: 2208–2214, 1998.
274. LIEBERMAN AP AND FISCHBECK KH. Triplet repeat expansion in neuromuscular disease. *Muscle Nerve* 23: 843–850, 2000.
275. LIN L AND GHOSH S. A glycine-rich region in NF-kappaB p105 functions as a processing signal for the generation of the p50 subunit. *Mol Cell Biol* 16: 2248–2254, 1996.
276. LISZTWAN J, IMBERT G, WIRBELAUER C, GSTAIGER M, AND KREK W. The von Hippel-Lindau tumor suppressor protein is a component of an E3 ubiquitin-protein ligase activity. *Genes Dev* 13: 1822–1833, 1999.
277. LODA M, CUKOR B, TAM SW, LAVIN P, FIORENTINO M, DRAETTA GF, JESSUP JM, AND PAGANO M. Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggresive colorectal carcinomas. *Nature Med* 3: 231–234, 1997.
278. LOEWE J, STOCK D, JAP B, ZWICKL P, BAUMEISTER W, AND HUBER R. Crystal structure of the 20S proteasome from the archeon *T. acidophilum* at 3.4 Angstrom resolution. *Science* 268: 533–539, 1995.
279. LORD JM, DAVEY J, FRIGERIO L, AND ROBERTS LM. Endoplasmic reticulum-associated protein degradation. *Semin Cell Dev Biol* 11: 159–164, 2000.
280. LORICK KL, JENSEN JP, FANG S, ONG AM, HATAKAYAMA S, AND WEISSMAN AM. RING finger mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc Natl Acad Sci USA* 96: 11364–11369, 1999.
281. LUCCHIARI-HARTZ M, VAN ENDERT PM, LAUVAU G, MAIER R, MEYERHANS A, MANN D, EICHMANN K, AND NIEDERMANN G. Cytotoxic T lymphocyte epitopes of HIV-1 Nef: generation of multiple definitive major histocompatibility complex class I ligands by proteasomes. *J Exp Med* 191: 239–252, 2000.
282. LUEDERS J, DEMAND J, AND HOEHFELD J. The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. *J Biol Chem* 275: 4613–4617, 2000.
283. LUPAS A, BAUMEISTER W, AND HOFMANN K. A repetitive sequence in subunits of the 26S proteasome and 20S cyclosome (APC). *Trends Biochem Sci* 22: 195–196, 1997.
284. LUPAS A, FLANAGAN JM, TAMURA T, AND BAUMEISTER W. Self compartmentalizing proteases. *Trends Biochem Sci* 22: 399–404, 1997.
285. LYAPINA S, COPE G, SHEVCHENKO A, SERINO G, TSUGE T, ZHOU C, WOLF DA, WEI N, SHEVCHENKO A, AND DESHAIES RJ. Promotion of NEDD-CUL1 conjugate cleavage by COP9 signalosome. *Science* 292: 1382–1385, 2001.
286. MA CP, SLAUGHTER CA, AND DEMARTINO GN. Identification, purification, and characterization of a protein activator (PA28) of the 20S proteasome. *J Biol Chem* 267: 10515–10523, 1992.
287. MADURA K, DOHMEN RJ, AND VARSHAVSKY A. *N*-recognin/Ubc2 interactions in the N-end rule pathway. *J Biol Chem* 268: 12046–12054, 1993.
288. MADURA K AND VARSHAVSKY A. Degradation of Gα by the N-end rule pathway. *Science* 265: 1454–1458, 1994.
289. MAHAJAN R, DELPHIN C, GUAN T, GERACE L, AND MELCHIOR F. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* 97–107, 1997.
290. MAMANE Y, HEYLBROECK C, GENIN P, ALGARTE M, SERVANT MJ, LEPAGE C, DELUCA C, KWON H, LIN R, AND HISCOTT J. IRFs: the next generation. *Gene* 237: 1–14, 1999.
291. MANIATIS T. A ubiquitin ligase complex essential for the NF-kB, Wnt/Wingless, and Hedgehog signaling pathways. *Genes Dev* 13: 505–514, 1999.
292. MANNHAUPT G, SCHNALL R, KARPOV V, VETTER I, AND FELDMANN H. Rpn4 acts as a transcription factor by binding to PACE, a nonamer box found upstream of 26S proteasomal and other genes in yeast. *FEBS Lett* 450: 27–34, 1999.
293. MARGOTTIN F, BOUR SP, DURAND H, SELIG L, BENICHOU S, RICHARD V, THOMAS D, STREBEL K, AND BENAROUS R. A novel human WD protein, human β-TrCP, that interacts with HIV-1 Vpu, connects CD4 to the ER degradation pathway through an F-box motif. *Mol Cell* 1: 565–574, 1998.
294. MARTI A, WIRBELAUER C, SCHEFFNER M, AND KREK W. Interaction between ubiquitin-protein ligase SCF-SKP2 and E2F-1 underlies the regulation of E2F-1 degradation. *Nature Cell Biol* 1: 14–19, 1999.
295. MASTRANDREA LD, YOU J, NILES EG, AND PICKART CM. E2/E3-mediated assembly of lysine 29-linked polyubiquitin chains. *J Biol Chem* 274: 27299–27306, 1999.
296. MATSUSHITA T AND SADLER JE. Identification of amino acid residues essential for von Willebrand factor binding to platelet glycoprotein Ib. Charged-to-alanine scanning mutagenesis of the A1 domain of human von Willebrand factor. *J Biol Chem* 270: 13406–13414, 1995.
297. MAUPIN-FURLOW JA AND FERRY JG. A proteasome from the methanogenic archaeon *Methanosarcina thermophila*. *J Biol Chem* 270: 28617–28622, 1995.
298. MAUPIN-FURLOW JA, WILSON HL, KACZOWKA SJ, AND OU MS. Proteasomes in the archaea: from structure to function. *Front Biosci* 5: D837–D865, 2000.
299. MAXWELL PH, WIESENER MS, CHANG GW, CLIFFORD SC, VAUX EG, COCKMAN ME, WYKOFF CC, PUGH CW, MAHER ER, AND RATCLIFFE PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 399: 271–275, 1999.
299a. MCCLELLAN AJ AND FRYDMAN J. Molecular chaperones and the art of recognizing a lost cause. *Nat Cell Biol* 3: E1–E3, 2001.
300. MCCUTCHEN-MALONEY SL, MATSUDA K, SHIMBARA N, BINNS DD, TANAKA K, SLAUGHTER CA, DEMARTINO GN, AND DE KLEIJN DP. cDNA cloning, expression, and functional characterization of PI31, a proline-rich inhibitor of the proteasome. *J Biol Chem* 275: 18557–18565, 2000.
301. MCGARRY TJ AND KIRSCHNER MW. Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell* 93: 1043–1053, 1998.
302. MCGRATH JP, JENTSCH S, AND VARSHAVSKY A. *UBA1*: an essential yeast gene encoding ubiquitin-activating enzyme. *EMBO J* 10: 227–236, 1991.
303. MEACHAM GC, PATTERSON C, WENYUE Z, YOUNGER JM, AND CYR DM. The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. *Nature Cell Biol* 3: 100–105, 2001.
304. MEIMOUN A, HOLTZMAN T, WEISSMAN Z, MCBRIDE HJ, STILLMAN DJ, FINK

GR, AND KORNITZER D. Degradation of the transcription factor Gcn4 requires the kinase Pho85 and the SCF(CDC4) ubiquitin-ligase complex. *Mol Biol Cell* 11: 915–927, 2000.
305. MELCHIOR F. Sumo nonclassical ubiquitin. *Annu Rev Cell Dev Biol* 16: 591–626, 2000.
306. MICHALEK MT, GRANT EP, GRAMM C, GOLDBERG AL, AND ROCK KL. A role for the ubiquitin-dependent proteolytic pathway in MHC class 1 restricted antigen presentation. *Nature* 363: 552–554, 1993.
307. MILLER JR AND MOON RT. Signal transduction through the β-catenin and specification of cell fate during embryogenesis. *Genes Dev* 10: 1443–1454, 1996.
308. MITCH WE AND GOLDBERG AL. Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. *N Engl J Med* 335: 1897–1905, 1996.
309. MIZUSHIMA N, NODA T, YOSHIMORI T, TANAKA Y, ISHII T, GEORGE MD, KLIONSKY DJ, OHSUMI M, AND OHSUMI Y. A protein conjugation system essential for autophagy. *Nature* 395: 395–398, 1998.
310. MOLINARI M AND MILNER J. p53 in complex with DNA is resistant to ubiquitin-dependent proteolysis in the presence of HPV-16 E6. *Oncogene* 10: 1849–1854, 1995.
311. MONTEL V, GARDRAT F, AZANZA JL, AND RAYMOND J. 20S proteasome, hsp90, p97 fusion protein, PA28 activator copurifying oligomers and ATPase activities. *Biochem Mol Biol Int* 47: 465–472, 1999.
312. MORI S, MURAKAMI-MORI K, AND BONAVIDA B. Interleukin-6 induces G_1 arrest through induction of $p27^{Kip1}$, a cyclin-dependent kinase inhibitor, and neuron-like morphology in LNCaP prostate tumor cells. *Biochem Biophys Res Commun* 257: 609–614, 1999.
312a. MORI S, TANAKA K, OMURA S, AND SAITO Y. Degradation process of ligand-stimulated platelet-derived growth factor β-receptor involves ubiquitin-proteasome proteolytic pathway. *J Biol Chem* 270: 29447–29452, 1995.
313. MULLER S AND SCHWARTZ LM. Ubiquitin in homeostasis, development and disease. *Bioessays* 17: 677–684, 1995.
314. MUNDT KE, PORTE J, MURRAY JM, BRIKOS C, CHRISTENSEN PU, CASPARI T, HAGAN IM, MILLAR JB, SIMANIS V, HOFMANN K, AND CARR AM. The COP9/signalosome complex is conserved in fission yeast and has a role in S phase. *Curr Biol* 9: 1427–1430, 1999.
315. MURAKAMI Y, MATSUFUJI S, HAYASHI SI, TANAHASHI N, AND TANAKA K. Degradation of ODC by the 26S proteasome. *Biochem Biophys Res Commun* 267: 1–6, 2000.
315a. MURATA S, MINAMI Y, MINAMI M, CHIBA T, AND TANAKA K. CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. *EMBO Rep* 2: 1133–1138, 2001.
316. NAKAGAWA K AND YOKASAWA H. Degradation of transcription factor IRF-1 by the ubiquitin-proteasome pathway. The C-terminal region governs protein stability. *Eur J Biochem* 267: 1680–1686, 2000.
317. NAKAMURA S, ROTH JA, AND MUKHOPADHYAY T. Multiple lysine mutations in the C-terminal domain of p53 interfere with Mdm2-dependent protein degradation and ubiquitination. *Mol Cell Biol* 20: 9391–9398, 2000.
317a. NASH P, TANG X, ORLICKY S, CHEN Q, GERTLER FB, MENDENHALL MD, SICHERI F, PAWSON T, AND TYERS M. Multisite phosphorylation of a CDK inhibitor sets a threshold for the onset of DNA replication. *Nature* 414: 514–521, 2001.
318. NAUMANN M, BECH-OTSCHIR D, HUANG X, FERRELL K, AND DUBIEL W. COP9 signalosome-directed c-Jun activation/stabilization is independent of JNK. *J Biol Chem* 274: 35297–35300, 1999.
318a. NAVON A AND GOLDBERG AL. Proteins are unfolded on the surface of the ATPase ring before transport into the proteasome. *Mol Cell* 8: 1339–1349, 2001.
319. NISHIZAWA M, FURUNO N, OKAZAKI K, TANAKA H, OGAWA Y, AND SAGATA N. Degradation of Mos by the N-terminal proline (Pro2)-dependent ubiquitin pathway on fertilization of *Xenopus* eggs: possible significance of natural selection for Pro2 in Mos. *EMBO J* 12: 4021–4027, 1993.
320. NISHIZAWA M, OKAZAKI K, FURUNO N, WATANABE N, AND SAGATA N. The "second-codon rule" and autophosphorylation govern the stability and activity of Mos during the meiotic cell cycle in *Xenopus* oocytes. *EMBO J* 11: 2433–2446, 1992.
321. NUBER U, SCHWARZ S, KAISER P, SCHNEIDER R, AND SCHEFFNER M. Cloning of human ubiquitin-conjugating enzymes UbcH6 and UbcH7 (E2-F1) and characterization of their interaction with E6-AP and RSP5. *J Biol Chem* 271: 2795–2800, 1996.
322. NUSSBAUM AK, DICK TP, KEILHOLZ W, SCHIRLE M, STEVANOVIC S, DIETZ K, HEINEMEYER W, GROLL M, WOLF DH, HUBER R, RAMMENSEE HG, AND SCHILD H. Cleavage motifs of the yeast 20S proteasome beta-subunits deduced from digests of enolase 1. *Proc Natl Acad Sci USA* 95: 12504–12509, 1998.
323. ODA H, KUMAR S, AND HOWLEY PM. Regulation of the Src family tyrosine kinase Blk through E6-AP-mediated ubiquitination. *Proc Natl Acad Sci USA* 96: 9557–9562, 1999.
323a. OGURA T AND WILKINSON AJ. AAA+ superfamily ATPases: common structure—diverse function. *Genes Cells* 6: 575–597, 2001.
324. OHBA M. Modulation of intracellular protein degradation by SSB1-SIS1 chaperon system in yeast *S. cerevisiae*. *FEBS Lett* 409: 307–311, 1997.
325. OKAZAKI K AND SAGATA N. The Mos/MAP kinase pathway stabilizes c-Fos by phosphorylation and augments its transforming activity in NIH 3T3 cells. *EMBO J* 14: 5048–5059, 1995.
326. ONISHI T, YAMAKAWA K, FRANCO OE, SUZUKI R, AND KAWAMURA J. $p27^{Kip1}$ is the key mediator of phenylacetate-induced cell cycle arrest in human prostate cancer cells. *Anticancer Res* 20: 3075–3081, 2000.
327. ORIAN A, GONEN H, BERCOVICH B, FAJERMAN I, EYTAN E, ISRAEL A, MERCURIO F, IWAI K, SCHWARTZ AL, AND CIECHANOVER A. SCF-β-TrCP ubiquitin ligase-mediated processing of NF-kB p105 requires phosphorylation of its C-terminus by IkB kinase. *EMBO J* 19: 2580–2591, 2000.
328. ORIAN A, SCHWARTZ AL, ISRAEL A, WHITESIDE S, KAHANA C, AND CIECHANOVER A. Structural motifs involved in ubiquitin-mediated processing of the NF-kappaB precursor p105: roles of the glycine-rich region and a downstream ubiquitination domain. *Mol Cell Biol* 19: 3664–3673, 1999.
329. ORTOLAN TG, TANGAONKAR P, LAMBERTSON D, CHEN L, SCHAUBER C, AND MADURA K. The DNA repair protein Rad23 is a negative regulator of multi-ubiquitin chain assembly. *Nat Cell Biol* 2: 601–607, 2000.
330. OSAKA F, SAEKI M, KATAYAMA S, AIDA N, TOH-E A, KOMINAMI K, TODA T, SUZUKI T, CHIBA T, TANAKA K, AND KATO S. Covalent modifier NEDD8 is essential for SCF ubiquitin-ligase in fission yeast. *EMBO J* 19: 3475–3484, 2000.
331. OSMULSKI PA AND GACZYNCKA M. A new large proteolytic complex distinct from the proteasome is present in the cytosol of fission yeast. *Curr Biol* 8: 1023–1026, 1998.
332. OSTERLUND MT, HARDTKE CS, WEI N, AND DENG XW. Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. *Nature* 405: 462–466, 2000.
333. PAGE AM AND HIETER P. The anaphase-promoting complex: new substrates and regulators. *Annu Rev Biochem* 68: 583–609, 1999.
334. PALOMBELLA VJ, RANDO OJ, GOLDBERG AL, AND MANIATIS T. The ubiquitin proteasome pathway is required for processing the NF-κB1 precursor protein and activation of NF-κB. *Cell* 78: 773–785, 1994.
335. PAPA FR, AMERIK AY, AND HOCHSTRASSER M. Interaction of the Doa4 deubiquitinating enzyme with the yeast 26S proteasome. *Mol Biol Cell* 10: 741–756, 1999.
336. PAPA FR AND HOCHSTRASSER M. The yeast *DOA4* gene encodes a deubiquitinating enzyme related to a product of the tre-2 oncogene. *Nature* 366: 313–319, 1993.
337. PATEL S AND LATTERICH M. The AAA team: related ATPases with diverse functions. *Trends Cell Biol* 8: 65–71, 1998.
338. PAUSE A, PETERSON B, SCHAFFAR G, STEARMAN R, AND KLAUSNER RD. Studying interactions of four proteins in the yeast two-hybrid system: structural resemblance of the pVHL/elongin BC/hCUL-2 complex with the ubiquitin ligase complex SKP1/cullin/F-box protein. *Proc Natl Acad Sci USA* 96: 9533–9538, 1999.
339. PAYNE AS, KELLY EJ, AND GITLIN JD. Functional expression of the Wilson disease protein reveals mislocalization and impaired copper-dependent trafficking of the common H1069Q mutation. *Proc Natl Acad Sci USA* 95: 10854–10859, 1998.
340. PAZ P, BROUWENSTIJN N, PERRY R, AND SHASTRI N. Discrete proteolytic intermediates in the MHC class I antigen processing pathway and MHC I-dependent peptide trimming in the ER. *Imunity* 11: 241–251, 1999.
341. PERRY WL, HUSTAD CM, SWING DA, O'SULLIVAN N, JENKINS NA, AND COPELAND NG. The itchy locus encodes a novel ubiquitin-protein ligase that is disrupted in a18H mice. *Nat Genet* 18: 143–146, 1998.
342. PETERS JM, FRANKE WW, AND KLEINSCHMIDT JA. Distinct 19S and 20S subcomplexes of the proteasome and their distribution in the nucleus and the cytoplasm. *J Biol Chem* 269: 7709–7718, 1994.

343. PETERS J-M, HARRIS JR, AND FINLEY D. *Ubiquitin and the Biology of the Cell.* New York: Plenum, 1998, p. 1–472.
344. PETERS JM, KING RW, HOOG C, AND KIRSCHNER MW. Identification of BIME as a subunit of the anaphase-promoting complex. *Science* 274: 1199–1201, 1996.
345. PFEIFER G, GEIER E, WILM M, LUCCHIARI-HARTZ M, BAUMEISTER W, EICHMANN K, AND NIEDERMANN G. A giant protease with potential to substitute for some functions of the proteasome. *Science* 283: 978–981, 1999.
346. PFLEGER CM AND KIRSCHNER MW. The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1. *Genes Dev* 14: 655–665, 2000.
347. PHAM A-D AND SAUER F. Ubiquitin-activating/conjugating activity of $TAF_{II}250$, a mediator of activation of gene expression in *Drosophila*. *Science* 289: 2357–2360, 2000.
348. PHILLIPS JB, WILLIAMS AJ, ADAMS J, ELLIOTT PJ, AND TORTELLA FC. Proteasome inhibitor PS519 reduces infarction and attenuates leukocyte infiltration in a rat model of focal cerebral ischemia. *Stroke* 31: 1686–1693, 2000.
349. PICKART CM. Ubiquitin in chains. *Trends Biochem Sci* 25: 544–548, 2000.
350. PICKART CM. Mechanisms underlying ubiquitination. *Annu Rev Biochem* 70: 503–533, 2001.
351. PICKART CM AND ROSE IA. Functional heterogeneity of ubiquitin carrier proteins. *J Biol Chem* 260: 1573–1581, 1985.
352. PLEMPER RK, BOHMLER S, BORDALLO J, SOMMER T, AND WOLF DH. Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation. *Nature* 388: 891–895, 1997.
353. PLEMPER RK AND WOLF DH. Retrograde protein translocation: ERADication of secretory proteins in health and disease. *Trends Biochem Sci* 24: 266–270, 1999.
354. PODUST VN, BROWNELL JE, GLADYSHEVA TB, LUO RS, WANG C, COGGINS MB, PIERCE JW, LIGHTCAP ES, AND CHAU V. A NEDD8 conjugation pathway is essential for proteolytic targeting of p27Kip1 by ubiquitination. *Proc Natl Acad Sci USA* 97: 4579–4584, 2000.
355. POLAKIS P. Wnt signaling and cancer. *Genes Dev* 14: 1837–1851, 2000.
356. PRINCIOTTA MF, SCHUBERT U, CHEN W, BENNINK JR, MYUNG J, CREWS CM, AND YEWDELL JW. Cells adapted to proteasome inhbitor 4-hydroxy-5-iodo-3-nitrophenylacetyl-Leu-Leu-Leucinal-vinyl sulfone require enzymatically active proteasomes for continued survival. *Proc Natl Acad Sci USA* 98: 513–518, 2001.
357. QIN S, NAKAJIMA B, NOMURA M, AND ARFIN SM. Cloning and characterization of a *Saccharomyces cerevisiae* gene encoding a new member of the ubiquitin-conjugating protein family. *J Biol Chem* 266: 15549–15554, 1991.
358. QIU L, JOAZEIRO N, FANG N, WANG HY, ELLY C, ALTMAN Y, FANG D, HUNTER V, AND LIU YC. Recognition and ubiquitination of Notch by Itch, a Hect-type E3 ubiquitin ligase. *J Biol Chem* 275: 35734–35737, 2000.
358a. RABINOVICH E, KEREM A, FROHLICH KU, DIAMANT N, AND BAR-NUN S. AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. *Mol Cell Biol* 22: 626–634, 2002.
359. RAMOS PC, HOCKENDORFF J, JOHNSON ES, VARSHAVSKY A, AND DOHMEN RJ. Ump1p is required for proper maturation of the 20S proteasome and becomes its substrate upon completion of the assembly. *Cell* 92: 489–499, 1998.
360. RAO H, UHLMANN F, NASMYTH K, AND VARSHAVSKY A. Degradation of cohesin subunit by the N-end rule pathway is essential for chromosome stability. *Nature* 410: 955–959, 2000.
360a. RAPE M, HOPPE T, GORR I, KALOCAY M, RICHLY H, AND JENTSCH S. Mobilization of processed, membrane-tethered SPT23 transcription factor by CDC48 (UFD1/NPL4), a ubiquitin-selective chaperone. *Cell* 107: 667–677, 2001.
361. RAVID T, DOOLMAN R, AVNER R, HARATS D, AND ROITELMAN J. The ubiquitin proteasome pathway mediates the regulated degradation of the mammalian 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J Biol Chem* 275: 35840–35847, 2000.
362. REALINI C, JENSEN CC, ZHANG Z, JOHNSTON SC, KNOWLTON JR, HILL CP, AND RECHSTEINER M. Characterization of recombinant REGα, REGβ, and REGγ proteasome activators. *J Biol Chem* 272: 25483–25492, 1997.
363. RECHSTEINER M, HOFFMAN L, AND DUBIEL W. The multicatalytic and 26S proteases. *J Biol Chem* 268: 6065–6068, 1993.
364. RECHSTEINER M, REALINI C, AND USTRELL V. The proteasome activator 11S REG (PA28) and class I antigen presentation. *Biochem J* 345: 1–15, 2000.
365. REINSTEIN E, SCHEFFNER M, OREN M, CIECHANOVER A, AND SCHWARTZ AL. Degradation of the E7 human papillomavirus oncoprotein by the ubiquitin-proteasome system: targeting via ubiquitination of the N-terminal residue. *Oncogene* 19: 5944–5950, 2000.
366. REISS Y, HELLER H, AND HERSHKO A. Binding sites of ubiquitin-protein ligase: binding of ubiquitin-protein conjugates and of ubiquitin-carrier protein. *J Biol Chem* 264: 10378–10383, 1989.
367. REISS Y AND HERSHKO A. Affinity purification of ubiquitin-protein ligase on immobilized protein substrates: evidence for the existence of separate NH_2-terminal binding sites on a single enzyme. *J Biol Chem* 265: 3685–3690, 1990.
368. REISS Y, KAIM D, AND HERSHKO A. Specificity of binding of NH_2-terminal residue of proteins to ubiquitin-protein ligase: use of amino acid derivatives to characterize specific binding sites. *J Biol Chem* 263: 2693–2698, 1988.
369. RICHMOND C, GORBEA C, AND RECHSTEINER M. Specific interactions between ATPase subunits of the 26S proteasome. *J Biol Chem* 272: 13403–13411, 1997.
370. ROCK KL AND GOLDBERG AL. Degradation of cell proteins and the generation of MHC class I-presented peptides. *Annu Rev Immunol* 17: 739–779, 1999.
371. ROEST HP, VAN KLAVEREN J, DE WIT J, VAN GURP CG, KOKEN MH, VERMEY M, VAN ROIJEN JH, HOOGERBRUGGE JW, VREEBURG JT, BAARENDS WM, BOOTSMA D, GROOTEGOED JA, AND HOEIJMAKERS JH. Inactivation of the HR6B ubiquitin-conjugating DNA repair enzyme in mice causes male sterility associated with chromatin modification. *Cell* 86: 799–810, 1996.
372. ROGERS S, WELLS R, AND RECHSTIENER M. Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science* 234: 364–368, 1986.
373. ROHRWILD M, PFEIFER G, SANTARIUS U, MUELLER SA, HAUANG HC, ENGEL A, BAUMEISTER W, AND GOLDBERG AL. The ATP-dependent HslV/U protease from *Escherichia coli* is a four-ring structure resembling the proteasome. *Nature Struct Biol* 4: 133–139, 1997.
374. ROSS CA, WOOD JD, SCHILLING G, PETERS MF JR, NUCIFORA FC, COOPER JK, SHARP AH, MARGOLIS RL, AND BORCHELT DR. Polyglutamine pathogenesis. *Philos Trans R Soc Lond B Biol Sci* 354: 1005–1011, 1999.
375. ROTH AF AND DAVIS NG. Ubiquitination of the yeast α-factor receptor. *J Cell Biol* 134: 661–674, 1996.
376. ROTIN D. Regulation of the epithelial sodium channel (ENaC) by accessory proteins. *Curr Opin Nephrol Hypertens* 9: 529–534, 2000.
377. RUBIN DM, GLICKMAN MH, LARSEN CN, DHRUVAKUMAR S, AND FINLEY D. Active site mutants in the six regulatory particle ATPases reveal multiple roles for ATP in the proteasome. *EMBO J* 17: 4909–4919, 1998.
378. RUBINFELD B, ROBBINS P, EL-GAMIL M, ALBERT I, PORFIRI E, AND POLAKIS P. Stabilization of b-catenin by genetic defects in melanoma cell lines. *Science* 275: 1790–1792, 1997.
379. RUEPP A, ECKERSKORN C, BOGYO M, AND BAUMEISTER W. Proteasome function is dispensable under normal but not under heat shock conditions in *Thermoplasma acidophilum*. *FEBS Lett* 425: 87–90, 1998.
380. RUSSELL SJ, REED SH, HUANG W, FRIEDBERG EC, AND JOHNSTON SJ. The 19S regulatory complex of the proteasome functions independently of proteolysis in nucleotide excision repair. *Mol Cell* 3: 687–695, 1999.
381. RUSSELL SJ, STEGER KA, AND JOHNSTON SA. Subcellular localization stoichiometry and protein levels of 26S proteasome subunits in yeast. *J Biol Chem* 274: 21943–21952, 1999.
382. SAEKI Y, TOH-E A, AND YOKOSAWA H. Rapid isolation and characterization of the yeast proteasome regulatory complex. *Biochem Biophys Res Commun* 273: 509–515, 2000.
383. SAIGOH K, WANG YL, SUH JG, YAMANISHI T, SAKAI Y, KIYOSAWA H, HARADA T, ICHIHARA N, WAKANA S, KIKUCHI T, AND WADA K. Intragenic deletion in the gene encoding ubiquitin carboxy-terminal hydrolase in gad mice. *Nat Genet* 23: 47–51, 1999.
384. SATOH K, SASAJIMA H, NYOUMURA KI, YOKOSAWA H, AND SAWADA H. Assembly of the 26S proteasome is regulated by phosphorylation of the p45/Rpt6 ATPase subunit. *Biochemistry* 40: 314–319, 2001.
385. SAURIN AJ, BORDEN KLB, BODDY MN, AND FREEMONT PS. Does this have a familiar RING? *Trends Biochem Sci* 21: 208–214, 1996.
386. SCHAUBER C, CHEN L, TONGAONAKAR P, VEGA I, LAMBERTSON D, POTTS

W, AND MADURA K. Rad23 links DNA repair to the ubiquitin/proteasome pathway. *Nature* 391: 715–718, 1998.
387. SCHAUBER C, CHEN L, TONGAONKAR P, VEGA I, AND MADURA K. Sequence elements that contribute to the degradation of yeast Gα. *Genes Cells* 3: 307–319, 1998.
388. SCHEFFNER M, HUIBREGSTE J, VIERSTRA RD, AND HOWLEY PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell* 75: 495–505, 1993.
389. SCHEFFNER M, NUBER U, AND HUIBREGSTE JM. Protein ubiquitination involving an E1–E2-E3 enzyme ubiquitin thioester cascade. *Nature* 373: 81–83, 1995.
390. SCHEFFNER M, WERNESS BA, HUIBREGTSE JM, LEVINE AL, AND HOWLEY PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 63: 1129–1136, 1990.
391. SCHERER DC, BROCKMAN JA, CHEN Z, MANIATIS T, AND BALLARD DW. Signal-induced degradation of IkBα requires site-specific ubiquitination. *Proc Natl Acad Sci USA* 92: 11259–11263, 1995.
392. SCHMIDTKE G, EMCH S, GROETTRUP M, AND HOLZHUTTER HG. Evidence for the existence of a non-catalytic modifier site of peptide hydrolysis by the 20S proteasome. *J Biol Chem* 275: 22056–22063, 2000.
393. SCHUBERT U, ANTON LC, GIBBS J, NORBURY CC, YEWDELL JW, AND BENNINK JR. Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* 404: 770–774, 2000.
394. SCHULE T, ROSE M, ENTIAN KD, THUMM M, AND WOLF DH. Ubc8p functions in catabolite degradation of fructose-1,6-bisphosphatase in yeast. *EMBO J* 19: 2161–2167, 2000.
395. SCHWAB M, LUTUM AS, AND SEUFERT W. Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. *Cell* 90: 683–693, 1997.
395a. SCHWAB M, NEUTZNER M, MOCKER D, AND SEUFERT W. Yeast Hct1 recognizes the mitotic cyclin Clb2 and other substrates of the ubiquitin ligase APC. *EMBO J* 20: 5165–5175, 2001.
396. SCHWARTZ AL, TRAUSCH JS, CIECHANOVER A, SLOT JW, AND GEUZE H. Immunoelectron microscopic localization of the ubiquitin-activating enzyme E1 in HepG2 cells. *Proc Natl Acad Sci USA* 89: 5542–5546, 1992.
396a. SCHWECHHEIMER C AND DENG X. COP9 signalosome revisited: a novel mediator of protein degradation. *Trends Cell Biol* 11: 420–426, 2001.
397. SCHWECHHEIMER C, SERINO G, CALLIS J, CROSBY WL, LYAPINA S, DESHAIES RJ, GRAY WM, ESTELLE M, AND DENG XW. Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCFTIRI in mediating auxin response. *Science* 292: 1379–1382, 2001.
398. SCHWOB E, BOEHM T, MENDENHALL MD, AND NASMYTH K. The B-type cyclin kinase inhibitor p40-SIC1 controls the G_1 to S transition in *S. cerevisiae*. *Cell* 79: 233–244, 1994.
399. SEARS C, OLESEN J, RUBIN DM, FINLEY D, AND MANIATIS T. NF-kB p105 processing via the ubiquitin-proteasome pathway. *J Biol Chem* 273: 1409–1419, 1998.
400. SEEGER M, FERRELL K, FRANK R, AND DUBIEL W. HIV-1 Tat inhibits the 20S proteasome and its 11S regulator-mediated activation. *J Biol Chem* 272: 8145–8148, 1997.
400a. SEEGER M, GORDON C, AND DUBIEL W. Protein stability: the COP9 signalosome gets in on the act. *Curr Biol* 11: R643–R646, 2001.
401. SEEGER M, KRAFT R, FERREL K, BECH-OTSCHIR D, DUMDEY R, SCHADE R, GORDON C, NAUMANN M, AND DUBIEL W. A novel protein complex involved in signal transduction possessing similarities to the 26S proteasome subunits. *FASEB J* 12: 469–478, 1998.
402. SERINO G, TSUGE T, KWOK SF, MATSUI M, WEI N, AND DENG XW. Arabidopsis cop8 and fus4 mutations define the same locus that encodes subunit 4 of the COP9 signalosome. *Plant Cell* 11: 1967–1980, 1999.
403. SEUFERT W AND JENTSCH S. Ubiquitin-conjugating enzymes UBC4 and UBC5 mediate selective degradation of short-lived and abnormal proteins. *EMBO J* 9: 543–550, 1990.
404. SEUFERT W, MCGRATH JP, AND JENTSCH S. UBC1 encodes a novel member of an essential subfamily of yeast ubiquitin-conjugating enzymes involved in protein degradation. *EMBO J* 9: 4535–4541, 1991.
405. SHAMU CE, STORY CM, RAPOPORT TA, AND PLOEGH HL. The pathway of US11-dependent degradation of MHC class I heavy chains involves a ubiquitin-conjugated intermediate. *J Cell Biol* 147: 45–58, 1999.
406. SHEAFF RJ, SINGER JD, SWANGER J, SMITHERMAN M, ROBERTS JM, AND CLURMAN BE. Proteasomal turnover of p21Cip1 does not require p21Cip1 ubiquitination. *Mol Cell* 5: 403–410, 2000.
407. SHEFLIN L, KEEGAN B, ZHANG W, AND SPAULDING SW. Inhibiting proteasomes in human HepG2 and LNCaP cells increases endogenous androgen receptor levels. *Biochem Biophys Res Commun* 276: 144–150, 2000.
408. SHERMAN MY AND GOLDBERG AL. Cellular defense mechanisms against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. *Neuron* 29: 15–32, 2001.
409. SHERR C AND ROBERTS J. Cdk inhibitors: positive and negative regulators of G_1-phase progression. *Genes Dev* 13: 1501–1514, 1999.
410. SHIH SC, SLOPER-MOULD KE, AND HICKE L. Monoubiquitin carries a novel internalization signal that is appended to activated receptors. *EMBO J* 19: 187–198, 2000.
411. SHIMURA H, HATTORI N, KUBO SI, MIZUNO Y, ASAKAWA S, MINOSHIMA S, SHIMIZU N, IWAI TC, K, TANAKA K, AND SUZUKI T. Familial Parkinson disease gene product, Parkin, is a ubiquitin-protein ligase. *Nat Genet* 25: 302–305, 2000.
412. SILVA CL, PORTARO FC, BONATO VL, DECAMARGO AC, AND FERRO ES. Thimet oligopeptidase (EC 3.4.24.15), a novel protein on the route of MHC class I antigen presentation. *Biochem Biophys Res Commun* 255: 591–595, 1999.
413. SKOWYRA D, KOEPP DM, KAMURA T, CONRAD NM, CONAWAY RC, CONAWAY JW, ELLEDGE SJ, AND HARPER JW. Reconstitution of G_1 Cyclin ubiquitination with complexes containing SCF^{Grr1} and Rbx1. *Science* 284: 662–665, 1999.
414. SLINGERLAND J AND PAGANO M. Regulation of the CDK inhibitor p27 and its deregulation in cancer. *J Cell Physiol* 183: 10–17, 2000.
415. SLOPER-MOULD KE, JEMC JC, PICKART CM, AND HICKE L. Distinct functional surface regions on ubiquitin. *J Biol Chem*. In press.
416. SOMMER T AND JENTSCH S. A protein translocation defect linked to ubiquitin conjugation at the endoplasmic reticulum. *Nature* 365: 176–179, 1993.
417. SPENCE J, GALI RR, DITTMAR G, SHERMAN F, KARIN M, AND FINLEY D. Cell cycle-regulated modification of the ribosome by a variant multiubiquitin chain. *Cell* 102: 67–76, 2000.
418. SPENCE J, SADIS S, HAAS AL, AND FINLEY D. A ubiquitin mutant with specific defects in DNA repair and multiubiquitination. *Mol Cell Biol* 15: 1265–1273, 1995.
419. SPIEGELMAN VS, SLAGA TJ, PAGANO M, MINAMOTO T, RONAI Z, AND FUCHS SY. Wnt/beta-catenin signaling induces the expression and activity of beta-TrCP ubiquitin ligase receptor. *Mol Cell* 5: 877–872, 2000.
420. STEINER H, CAPELL A, PESOLD B, CITRON M, KLOETZEL PM, SELKOE DJ, ROMIG H, MENDLA K, AND HAASS C. Expression of Alzheimer's disease-associated Presenilin-1 is controlled by proteolytic degradation and complex formation. *J Biol Chem* 273: 32322–32331, 1998.
421. STENOIEN DL, CUMMINGS CJ, ADAMS HP, MANICINI MG, PATEL K, DEMARTINO GN, MARCELLI M, WEIGEL NL, AND MANCINI MA. Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. *Hum Mol Gen* 8: 731–741, 1999.
422. STEPHEN AG, TRAUSCH-AZAR JS, CIECHANOVER A, AND SCHWARTZ AL. The ubiquitin-activating enzyme E1 is phosphorylated and localized to the nucleus in a cell cycle-dependent manner. *J Biol Chem* 271: 15608–15614, 1996.
423. STOHWASSER R, SALZMANN U, RUPPERT T, KLOETZEL PM, AND HOLZHUETTER HG. Kinetic evidences for facilitation of peptide channeling by the proteasomal activator PA28. *Eur J Biochem* 267: 6221–6230, 2000.
424. STOLTZE L, NUSSBAUM AK, SIJTS A, EMMERICH NP, KLOETZEL PM, AND SCHILD H. The function of the proteasome system in MHC class I antigen processing. *Immunol Today* 21: 317–319, 2000.
425. STRICKLAND E, HAKALA K, THOMAS PJ, AND DEMARTINO GN. Recognition of misfolding proteins by PA700, the regulatory subcomplex of the 26S proteasome. *J Biol Chem* 275: 5565–5572, 2000.
426. STROUS GJ AND GOVERS R. The ubiquitin-proteasome system and endocytosis. *J Cell Sci* 112: 1417–1423, 1999.
427. SWAMINATHAN S, AMERIK AY, AND HOCHSTRASSER M. The Doa4 deubiquitinating enzyme is required for ubiquitin homeostasis in yeast. *Mol Biol Cell* 10: 2583–2594, 1999.
427a. SWANSON R, LOCHER M, AND HOCHSTRASSER M. A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and MATα2 repressor degradation. *Genes Dev* 15: 2660–2674, 2001.
428. TAKEUCHI J, FUJIMURO M, YOKOSAWA H, TANAKA K, AND TOH-E A. Rpn9

is required for efficient assembly of the yeast 26S proteasome. *Mol Cell Biol* 10: 6575–6584, 1999.
429. TAMURA N, LOTTSPEICH F, BAUMEISTER W, AND TAMURA T. The role of tricorn protease and its aminopeptidase-interacting factors in cellular protein degradation. *Cell* 95: 637–648, 1998.
430. TAN P, FUCHS SY, CHEN A, WU K, GOMEZ C, RONAI Z, AND PAN Z-Q. Recruitment of a Roc1-Cul1 ubiquitin ligase by Skp1 and HOS to catalyze the ubiquitination of IkBα. *Mol Cell* 3: 527–533, 1999.
431. TANAHASHI N, MURAKAMI Y, MINAMI Y, SHIMBARA N, HENDIL KB, AND TANAKA K. Hybrid proteasomes: induction by interferon-gamma and contribution to ATP-dependent proteolysis. *J Biol Chem* 275: 14336–14345, 2000.
432. TATEBE H AND YANAGIDA M. Cut8, essential for anaphase, controls localization of 26S proteasome, facilitating destruction of cyclin and Cut2. *Curr Biol* 10: 1329–1338, 2000.
432a. TATEISHI K, OMATA M, TANAKA K, AND CHIBA T. The NEDD8 system is essential for cell cycle progression and morphogenetic pathway in mice. *J Cell Biol* 155: 571–577, 2001.
433. TERRELL J, SHIH SC, DUNN R, AND HICKE L. A function for monoubiquitination in the internalization of a G protein-coupled receptor. *Mol Cell* 1: 193–202, 1998.
434. THIRUMALAI D AND LORIMER GH. Chaperonin-mediated protein folding. *Annu Rev Biophys Biomol Struct* 30: 245–269, 2001.
435. THROWER JS, HOFFMAN L, RECHSTEINER M, AND PICKART C. Recognition of the polyubiquitin proteolytic signal. *EMBO J* 19: 94–102, 2000.
436. TOMODA K, KUBOTA Y, AND KATO J. Degradation of the cyclin dependent kinase inhibitor p27/Kip1 is instigated by Jab1. *Nature* 398: 160–165, 1999.
437. TONE Y, TANAHASHI N, TANAKA K, FUJIMORO M, YOKOSAWA H, AND TOH-E A. Nob1, a new essential protein associates with the 26S proteasome of growing *S. cerevisiae* cells. *Gene* 243: 37–45, 2000.
438. TONGAONKAR P, CHEN L, LAMBERTSON D, KO B, AND MADURA K. Evidence for an interaction between ubiquitin-conjugating enzymes and the 26S proteasome. *Mol Cell Biol* 2000: 4691–4698, 2000.
439. TOUITOU R, RICHARDSON J, BOSE S, NAKANISHI M, RIVETT J, AND ALLDAY MJ. A degradation signal located in the C-terminus of p21WAF1/CIP1 is a binding site for the C8 alpha-subunit of the 20S proteasome. *EMBO J* 20: 2367–2375, 2001.
440. TOWNSLEY FM, ARISTARKHOV A, BECK S, HERSHKO A, AND RUDERMAN JV. Dominant-negative cyclin-selective ubiquitin carrier protein E2-C/UbcH10 blocks cells in metaphase. *Proc Natl Acad Sci USA* 94: 2362–2367, 1997.
441. TOWNSLEY FM AND RUDERMAN JV. Functional analysis of the *Saccharomyces cerevisiae* UBC11 gene. *Yeast* 14: 747–757, 1998.
442. TRAVERS KJ, PATIL CK, WODICKA L, LOCKHART DJ, WEISSMAN JS, AND WALTER P. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* 101: 249–258, 2000.
443. TREIER M, STASZEWSKI LM, AND BOHMANN D. Ubiquitin-dependent c-Jun degradation in vivo is mediated by the delta domain. *Cell* 78: 787–798, 1994.
444. TSIHLIAS J, KAPUSTA L, AND SLINGERLAND J. The prognostic significance of altered cyclin-dependent kinase inhibitors in human cancer. *Annu Rev Med* 50: 401–423, 1999.
445. TSVETKOV LM, YEH KH, LEE SJ, SUN H, AND ZHANG H. p27Kip1 ubiquitination and degradation is regulated by the SCF^{Skp2} complex through phosphorylated Thr187 in p27. *Curr Biol* 9: 661–664, 1999.
446. TUGENDREICH S, TOMKIEL J, EARNSHAW W, AND HIETER P. CDC27Hs colocalizes with CDC16Hs to the centrosome and mitotic spindle and is essential for the metaphase to anaphase transition. *Cell* 81: 261–268, 1995.
447. TURNER CT, DU F, AND VARSHAVSKY A. Peptides accelerate their uptake by activating ubiquitin-dependent proteolytic pathway. *Nature* 405: 579–583, 2000.
448. TURNER G AND VARSHAVSKY A. Detecting and measuring cotranslational protein degradation in vivo. *Science* 289: 2117–2120, 2000.
449. TYERS M AND WILLEMS AR. One RING to rule a superfamily of E3 ubiquitin ligases. *Science* 284: 901–904, 1999.
450. VANDERLUGT CL, RAHBE SM, ELLIOTT PJ, DAL-CANTO MC, AND MILLER SD. Treatment of established relapsing experimental autoimmune encephalomyelitis with the proteasome inhibitor PS-519. *J Autoimmun* 14: 205–211, 2000.
451. VAN LEEUWEN FW, DE KLEIJN DP, VAN DEN HURK HH, NEUBAUER A, SONNEMANS MA, SLUIJS JA, KOYCU S, RAMDJIELAL RDJ, SALEHI A, MARTENS GJM, GROSVELD FG, PETER J, BURBACH H, AND HOL EM. Frameshift mutants of β-amyloid precursor protein and ubiquitin-B in Alzheimer's and Down's patients. *Science* 279: 242–247, 1998.
452. VAN NOCKER S, DEVERAUX Q, RECHSTEINER M, AND VIERSTRA RD. *Arabidopsis MBP1* gene encodes a conserved ubiquitin recognition component of the 26S proteasome. *Proc Natl Acad Sci USA* 93: 856–860, 1996.
453. VAN NOCKER S, SADIS S, RUBIN DM, GLICKMAN MH, FU H, COUX O, WEFES I, FINLEY D, AND VIERSTRA RD. The multiubiquitin chain binding protein Mcb1 is a component of the 26S proteasome in *S. cerevisiae* and plays a nonessential, substrate-specific role in protein turnover. *Mol Cell Biol* 11: 6020–6028, 1996.
454. VARSHAVSKY A, TURNER G, DU F, AND XIE Y. The ubiquitin system and the N-end rule pathway. *Biol Chem* 381: 779–789, 2000.
455. VERMA R, ANNAN R, HUDDLESTON M, CARR S, REYNARD G, AND DESHAIES RJ. Phosphorylation of Sic1p by G_1 Cdk required for its degradation and entry into S phase. *Science* 278: 455–460, 1997.
455a. VERMA R, CHEN S, FELDMAN R, SCHIELTZ D, YATES J, DOHMEN J, AND DESHAIES RJ. Proteasomal proteomics: identification of nucleotide-sensitive proteasome-interacting proteins by mass spectrometric analysis of affinity-purified proteasomes. *Mol Biol Cell* 11: 3425–3439, 2000.
456. VISCIDI RP, MAYUR K, LEDERMAN HM, AND FRANKEL AD. Inhibition of antigen-induced lymphocyte proliferation by Tat protein from HIV-1. *Science* 246: 1606–1608, 1989.
457. VISINTIN R, PRINZ S, AND AMON A. CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. *Science* 278: 460–463, 1997.
458. VOGES D, ZWICKL P, AND BAUMEISTER W. The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu Rev Biochem* 68: 1015–1068, 1999.
459. WAGNER BJ AND MARGOLIS JW. Age-dependent association of isolated bovine lens multicatalytic proteinase complex (proteasome) with heat-shock protein 90, an endogenous inhibitor. *Arch Biochem Biophys* 323: 455–462, 1995.
460. WALTER J, URBAN J, VOLKWEIN K, AND SOMMER T. Sec61p-independent degradation of the tail-anchored ER membrane protein Ubc6p. *EMBO J* 20: 3124–3131, 2001.
461. WANG C, DENG L, HONG M, AKKARAJU GR, INOUE IJ, AND CHEN ZJ. Tak1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* 412: 346–351, 2001.
462. WANG EW, KESSLER BM, BORODOVSKY A, CRAVATT BF, BOGYO M, PLOEGH HL, AND GLAS R. Integration of the ubiquitin-proteasome pathway with a cytosolic oligopeptidase activity. *Proc Natl Acad Sci USA* 97: 9990–9995, 2000.
463. WANG G, WANG B, AND JIANG J. Protein kinase A antagonizes Hedgehog signaling by regulating both the activator and repressor forms of *Cubitus interruptus*. *Genes Dev* 13: 2828–2837, 1999.
464. WANG Y, GUTTOH DS, AND ANDROLEWICZ MJ. TAP prefers to transport melanoma antigenic peptides which are longer than the optimal T-cell epitope: evidence for further processing in the endoplasmic reticulum. *Melanoma Res* 8: 345–353, 1998.
465. WATANABE TK, SAITO A, SUZUKI M, FUJIWARA T, TAKAHASHI E, SLAUGHTER CA, DEMARTINO GN, HENDIL KB, CHUNG CH, TANAHASHI N, AND TANAKA K. cDNA cloning and characterization of a human proteasomal modulator subunit p27. *Genomics* 50: 241–250, 1998.
466. WEBER-BAN EU, REID BG, MIRANKER AD, AND HORWICH AL. Global unfolding of a substrate protein by the Hsp100 chaperone ClpA. *Nature* 401: 90–93, 1999.
467. WEI N, TSUGE T, SERINO G, DOHMAE N, TAKIO K, MATSUI M, AND DENG XW. The COP9 complex is conserved between plants and mammals and is related to the 26S proteasome regulatory complex. *Curr Biol* 8: 919–922, 1998.
468. WEISSMAN AM. Themes and variations on ubiquitylation. *Nature Rev Cell Mol Biol* 2: 169–179, 2001.
469. WHITBY FJ, MASTERS EI, KRAMER L, KNOWLTON JR, YAO Y, WANG C, AND HILL CP. Structural basis for the activation of 20S proteasomes by 11S regulators. *Nature* 408: 115–120, 2000.
470. WIEBEL FF AND KUNAU WH. The Pas2 protein essential for peroxisome biogenesis is related to ubiquitin-conjugating enzymes. *Nature* 359: 73–76, 1992.
471. WIGLEY WC, FABUNMI RP, LEE MG, MARINO CR, MUALLEM S, DE-

MARTINO GN, AND THOMAS PJ. Dynamic association of proteasomal machinery with the centrosome. *J Cell Biol* 145: 481–490, 1999.

471a. WILKINSON CR, SEEGER M, HARTMANN-PETERSEN R, STONE M, WALLACE M, SEMPLE C, AND GORDON C. Proteins containing the UBA domain are able to bind to multi-ubiquitin chains. *Nat Cell Biol* 3: 939–943, 2001.

472. WILKINSON CR, WALLACE M, MORPHEW M, PERRY P, ALLSHIRE R, JAVERZAT JP, MCINTOSH JR, AND GORDON C. Localization of the 26S proteasome during mitosis and meiosis in fission yeast. *EMBO J* 17: 6465–6476, 1998.

473. WILKINSON CRM, FERRELL K, PENNY M, WALLACE M, DUBIEL W, AND GORDON C. Analysis of a gene encoding Rpn10 of the fission yeast proteasome reveals that the polyubiquitin-binding site of this subunit is essential when Rpn12/Mts3 activity is compromised. *J Biol Chem* 275: 15182–15192, 2000.

474. WILKINSON KD. Ubiquitination and deubiquitination: targeting of proteins for degradation by the proteasome. *Semin Cell Dev Biol* 11: 141–148, 2000.

475. WILKINSON KD, TASHAYEV VL, OCONNOR LB, LARSEN CN, KASPEREL E, AND PICKART CM. Metabolism of the polyubiquitin degradation signal: structure, mechanism, and role of isopeptidase T. *Biochemistry* 34: 14535–14546, 1995.

476. WILSON HL, OU MS, ALDRICH HC, AND MAUPIN-FURLOW J. Biochemical and physical properties of the *M. jannaschii* 20S proteasome and PAN, a homolog of the ATPase (Rpt) subunits of the eucaryal 26S proteasome. *J Bacteriol* 186: 1680–1692, 2000.

477. WOLF S, NAGY I, LUPAS A, PFEIFER G, CEJKA Z, MUELLER SA, ENGEL A, DE MOT R, AND BAUMEISTER W. Characterization of ARC, a divergent member of the AAA ATPase family from *Rhodococcus erythropolis*. *J Mol Biol* 277: 13–25, 1998.

477a. WU K, CHEN A, TAN P, AND PAN ZQ. The Nedd8-conjugated ROC1-CUL1 core ubiquitin ligase utilizes Nedd8 charged surface residues for efficient polyubiquitin chain assembly catalyzed by Cdc34. *J Biol Chem* 277: 516–527, 2002.

478. XIE Y AND VARSHAVSKY A. The E2–E3 interaction in the N-end rule pathway: the RING-H2 finger of E3 is required for the synthesis of multiubiquitin chain. *EMBO J* 18: 6832–6844, 1999.

479. XIE Y AND VARSHAVSKY A. Physical association of ubiquitin ligases and the 26S proteasome. *Proc Natl Acad Sci USA* 97: 2497–2502, 2000.

480. XIE Y AND VARSHAVSKY A. RPN4 is a ligand, substrate, and transcriptional regulator of the 26S proteasome: a negative feedback circuit. *Proc Natl Acad Sci USA* 98: 3056–3061, 2001.

480a. XIRODIMAS DP, STEPHEN CW, AND LANE DP. Cocompartmentalization of p53 and Mdm2 is a major determinant for Mdm2-mediated degradation of p53. *Exp Cell Res* 270: 66–77, 2001.

481. YAMADA H, KUMADA K, AND YANAGIDA M. Distinct subunit functions and cell cycle regulated phosphorylation of 20S APC/cyclosome required for anaphase in fission yeast. *J Cell Sci* 110: 1793–1804, 1997.

482. YAMANO H, GANNON J, AND HUNT T. The role of proteolysis in cell cycle progression in *Schizosaccharomyces pombe*. *EMBO J* 15: 5268–5279, 1996.

483. YAMASHITA YM, NAKASEKO Y, SAMEJIMA I, KUMADA K, YAMADA H, MICHAELSON D, AND YANAGIDA M. 20S cyclosome complex formation and proteolytic activity inhibited by the cAMP/PKA pathway. *Nature* 384: 276–279, 1996.

483a. YANG Y, FANG S, JENSEN JP, WEISSMAN AM, AND ASHWELL JD. Ubiquitin-protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli. *Science* 288: 874–877, 2000.

484. YAO T AND COHEN RE. Giant proteases: beyond the proteasome. *Curr Biol* 9: R551–R553, 1999.

485. YAO Y, HUANG L, KRUTCHINSKY A, WONG ML, STANDING KG, BURLINGAME AL, AND WANG CC. Structural and functional characterizations of the proteasome-activating protein PA26 from *Trypanosoma brucei*. *J Biol Chem* 274: 33921–33930, 1999.

486. YARON A, GONEN H, ALKALAY I, HATZUBAI A, JUNG S, BEYTH S, MERCURIO F, MANNING AM, CIECHANOVER A, AND BEN-NERIAH Y. Inhibition of NF-kB cellular function via specific targeting of the IkB-ubiquitin ligase. *EMBO J* 16: 6486–6494, 1997.

487. YARON A, HATZUBAI A, DAVIS M, LAVON I, AMIT S, MANNING AM, ANDERSEN JS, MANN M, MERCURIO F, AND BEN-NERIAH Y. Identification of the receptor component of the IkB-ubiquitin ligase. *Nature* 396: 590–594, 1998.

487a. YE Y, MEYER HH, AND RAPOPORT TA. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 414: 652–656, 2001.

488. YEH ET, GONG L, AND KAMITANI T. Ubiquitin-like proteins: new wines in new bottles. *Gene* 248: 1–14, 2000.

489. YEWDELL JW AND BENNINK JR. Cut and trim: generating MHC class I peptide ligands. *Curr Opin Immunol* 13: 13–18, 2001.

490. YEWDELL JW, SCHUBERT U, AND BENNINK JR. At the crossroads of cell biology and immunology: DRiPs and other sources of peptide ligands for MHC class I molecules. *J Cell Sci* 114: 845–851, 2001.

491. YOU J AND PICKART CM. A HECT domain E3 enzyme assembles novel polyubiquitin chains. *J Biol Chem* 276: 19871–19878, 2001.

492. YOUNG P, DEVERAUX Q, BEAL RE, PICKART CM, AND RECHSTEINER M. Characterization of two polyubiquitin binding sites in the 26S protease subunit 5a. *J Biol Chem* 273: 5461–5467, 1998.

493. YU H, PETERS JM, KING RW, PAGE AM, HIETER P, AND KIRSCHNER MW. Identification of a cullin homology region in a subunit of the anaphase-promoting complex. *Science* 279: 1219–1222, 1998.

494. ZACHARIAE W, SCHWAB M, NASMYTH K, AND SEUFERT W. Control of cyclin ubiquitination by CDK-regulated binding of Hct1 to the anaphase promoting complex. *Science* 282: 1721–1724, 1998.

495. ZAISS DM, STANDERA S, HOLZHUTTER H, KLOETZEL P, AND SIJTS AJ. The proteasome inhibitor PI31 competes with PA28 for binding to 20S proteasomes. *FEBS Lett* 457: 333–338, 1999.

496. ZHANG H, KOBAYASHI R, GALAKTIONOV K, AND BEACH D. p19Skp1 and p45Skp2 are essential elements of the cyclin A-CDK2 S phase kinase. *Cell* 82: 915–925, 1995.

497. ZHANG Y, CHANG C, GEHLING DJ, HEMMATI-BRIVANLOU A, AND DERYNCK R. Regulation of Smad degradation and activity by Smurf2, an E3 ubiquitin ligase. *Proc Natl Acad Sci USA* 98: 974–979, 2001.

498. ZHANG Y, GAO J, CHUNG KK, HUANG H, DAWSON VL, AND DAWSON TM. Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. *Proc Natl Acad Sci USA* 97: 13354–13359, 2000.

499. ZHANG Z, TORII N, FURUSAKA A, MALAYAMAN N, HU Z, AND LIANG TJ. Structural and functional characterization of interaction between hepatitis B virus protein X and the proteasome complex. *J Biol Chem* 275: 15157–15165, 2000.

500. ZHENG N, WANG P, JEFFREY PD, AND PAVLETICH NP. Structure of a c-Cbl-UbcH7 complex: RING domain function in ubiquitin-protein ligases. *Cell* 102: 533–539, 2000.

501. ZHOU P AND HOWLEY PM. Ubiquitination and degradation of the substrate recognition subunits of SCF ubiquitin-protein ligases. *Mol Cell* 2: 571–580, 1998.

501a. ZHOU C, SEIBERT V, GEYER R, RHEE E, LYAPINA S, COPE G, DESHAIES RJ, AND WOLF DA. The fission yeast COP9/signalosome is involved in cullin modification by ubiquitin-related Ned8p. *BMC Biochem* 2: 7, 2001.

502. ZHU H, KAVSAK P, ABDOLLAH S, WRANA JL, AND THOMSEN GH. A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature* 400: 687–693, 1999.

503. ZWICKL P, BAUMEISTER W, AND STEVEN A. Dis-assembly lines: the proteasome and related ATPase-assisted proteases. *Curr Opin Struct Biol* 10: 242–250, 2000.

504. ZWICKL P, NG D, WOO KM, KLENK HP, AND GOLDBERG AL. An archaebacterial ATPase, homologous to ATPases in the eukaryotic 26S proteasome, activates protein breakdown by 20S proteasomes. *J Biol Chem* 274: 26008–26014, 1999.